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(54) **FGFR-FC FUSION PROTEINS AND THE USE THEREOF**

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A61K 38/00 (2006.01)

C07K 16/28 (2006.01)

(52) **U.S. Cl.**

CPC **C07K 16/2863** (2013.01); **A61K 47/48369**
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2319/30 (2013.01)

(58) **Field of Classification Search**

CPC C07K 14/71; C07K 2319/32; C07K
2319/30; C07K 2319/00

See application file for complete search history.

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(57) **ABSTRACT**

The present invention belongs to the field of biotechnology
and relates to the treatment of diseases, especially the treat-
ment of FGF overexpression-related diseases. Particularly,
the present invention relates to FGFR-Fc fusion proteins and
the use thereof in the treatment of angiogenesis regulation-
related diseases. More particularly, the present invention
relates to isolated soluble FGFR-Fc fusion proteins and their
applications in manufacture of the medicament for the treat-
ment of angiogenesis regulation-related diseases.

6 Claims, 8 Drawing Sheets

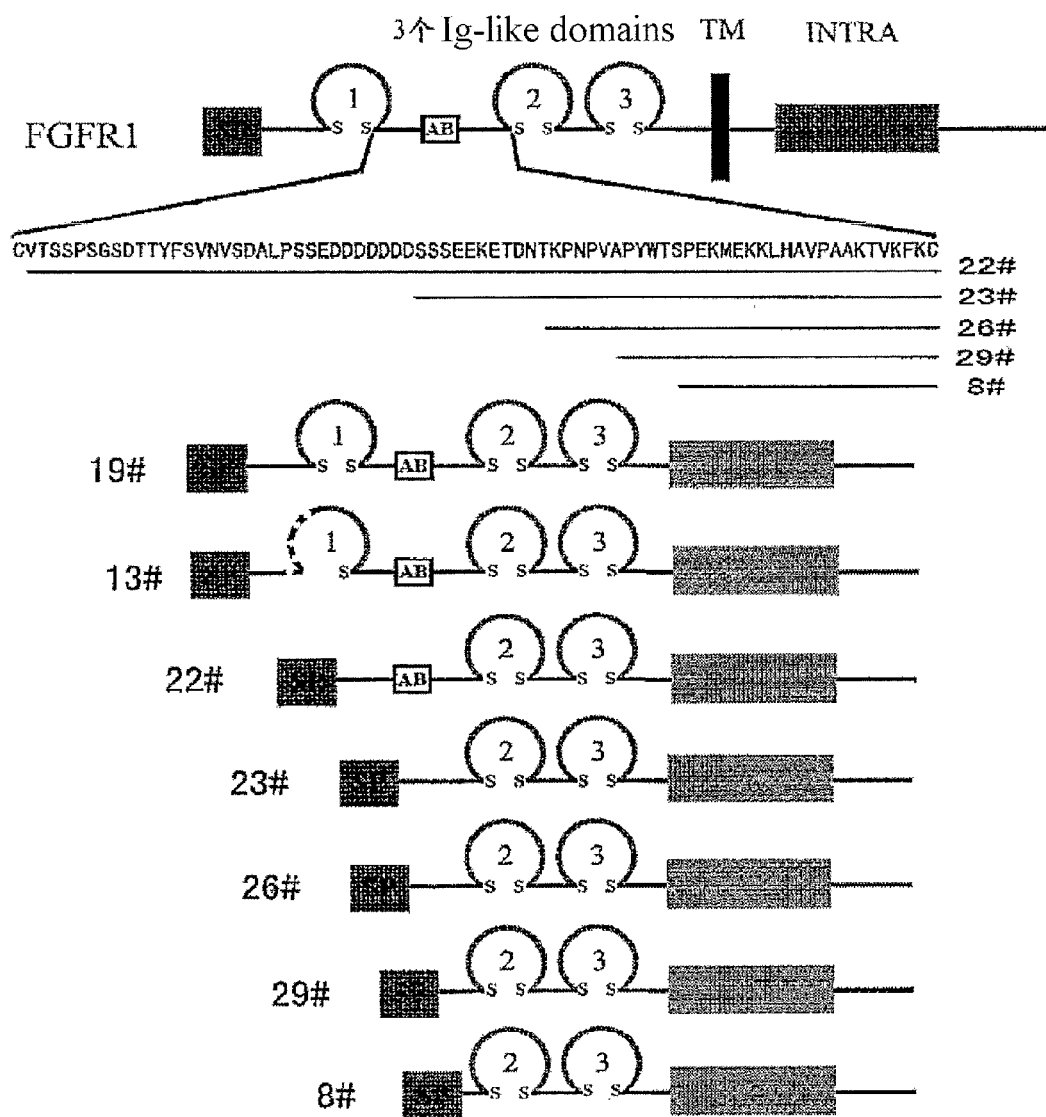


FIG. 1

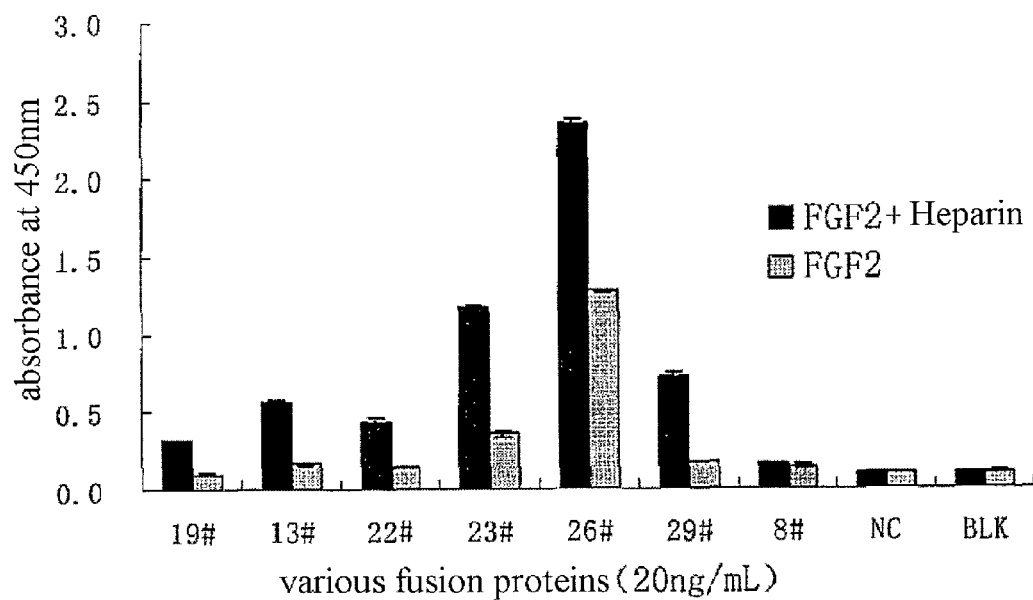


FIG. 2

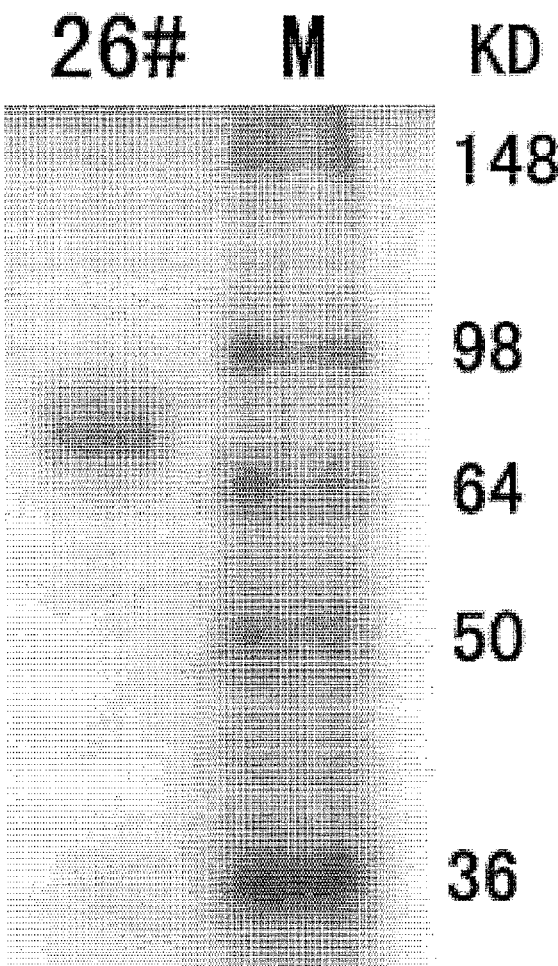


FIG. 3

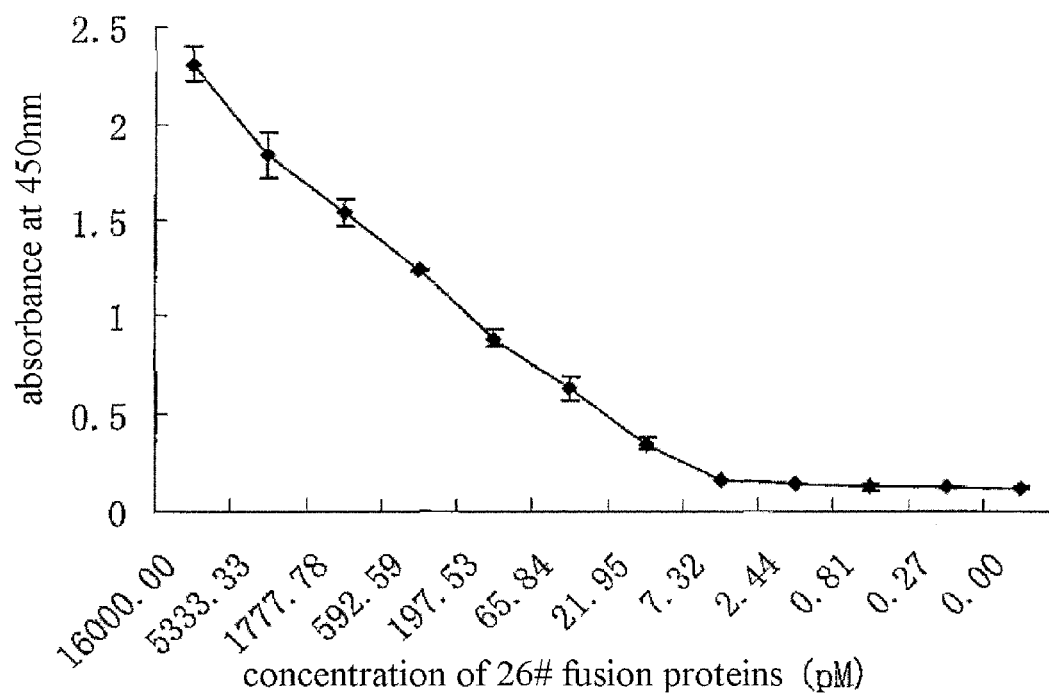


FIG. 4

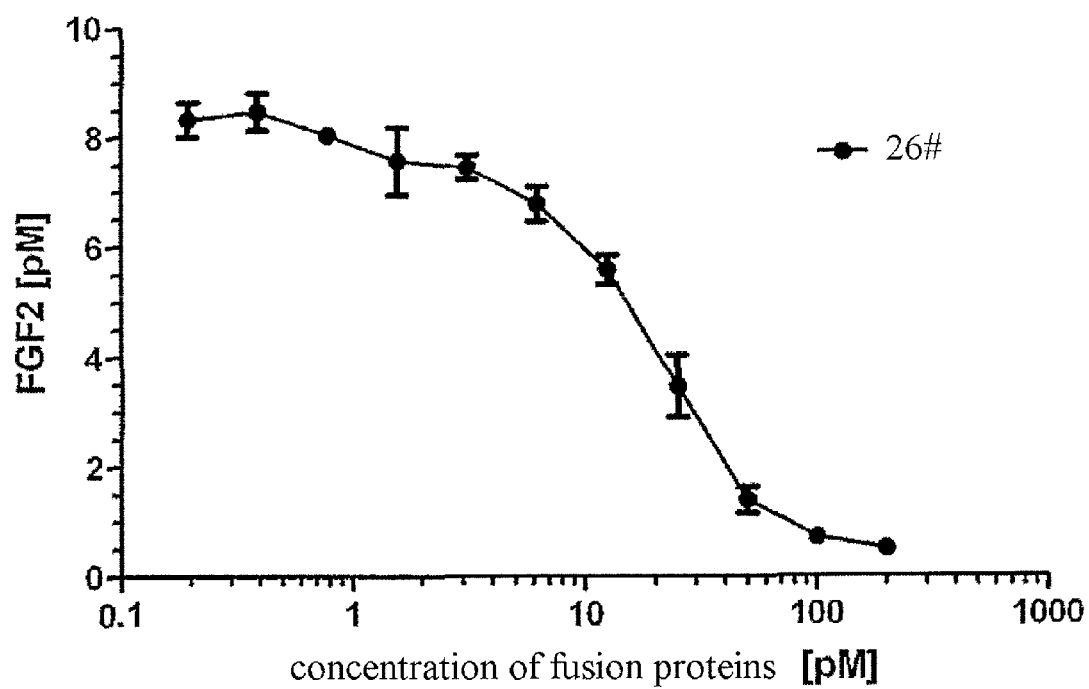


FIG. 5

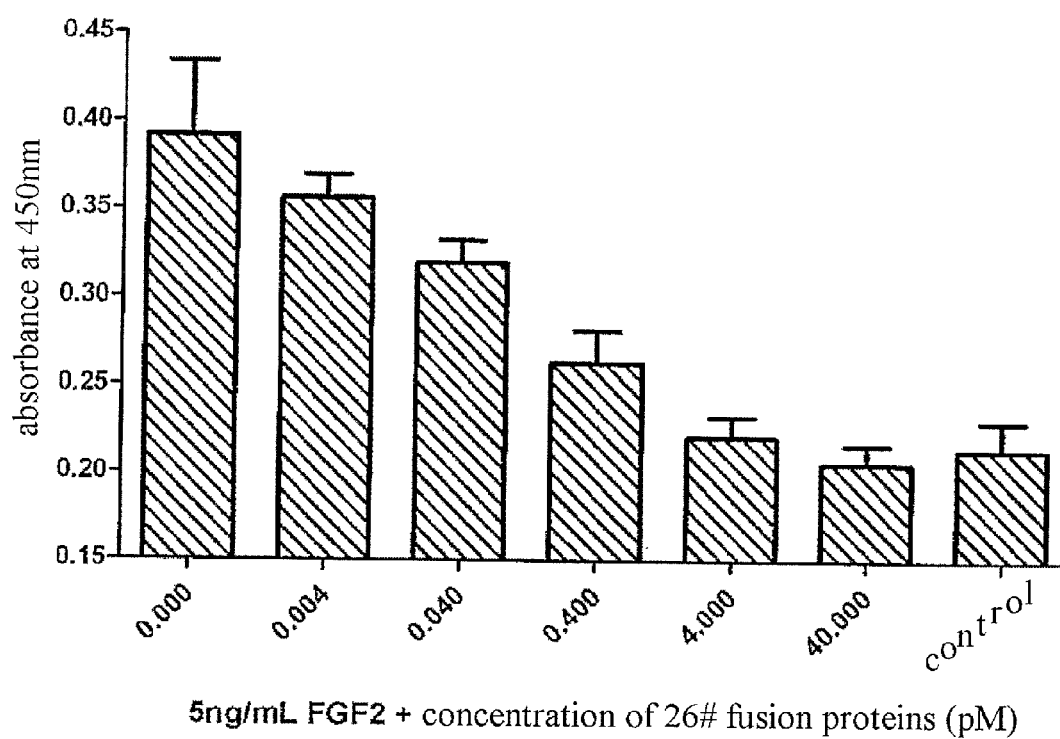


FIG. 6

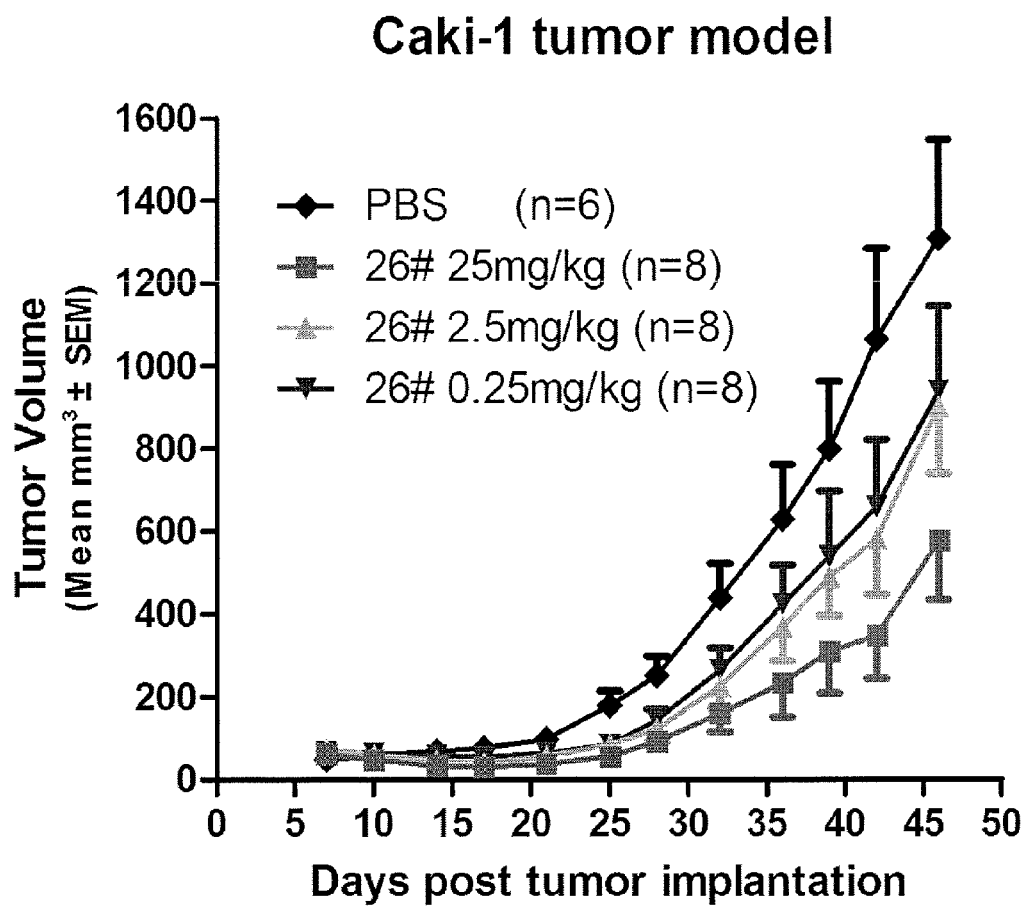


FIG. 7

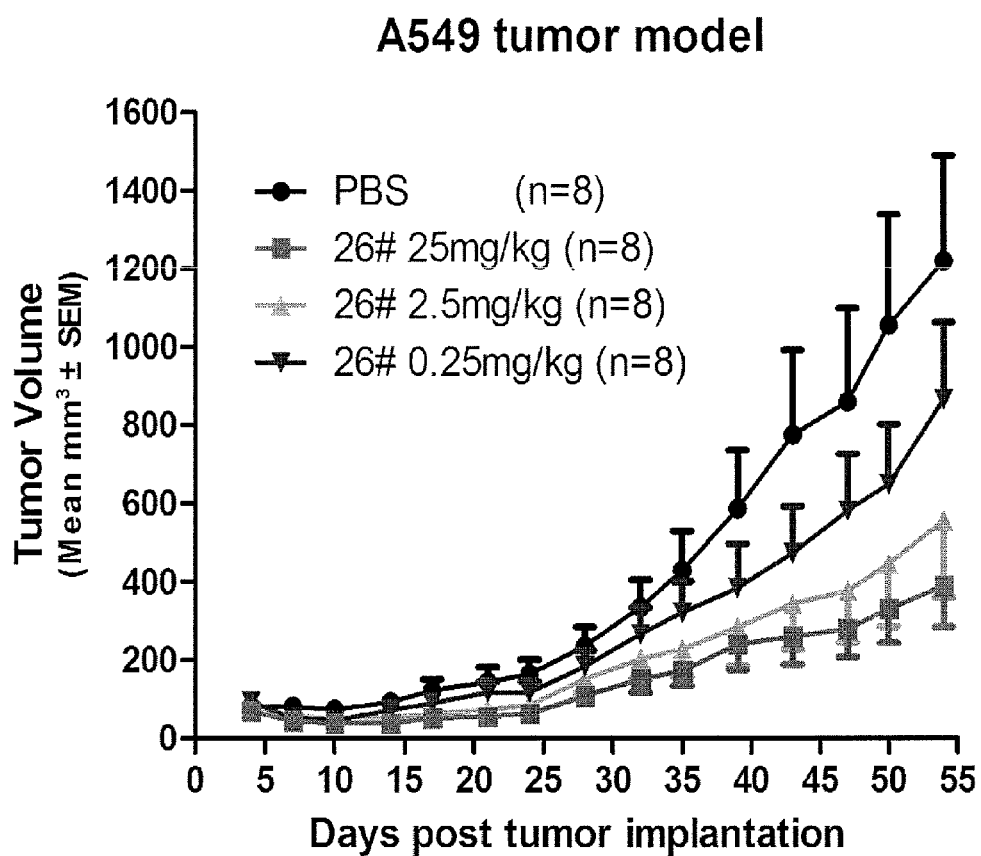


FIG. 8

FGFR-FC FUSION PROTEINS AND THE USE THEREOF

CROSS-REFERENCE TO A RELATED APPLICATION

The present application is a continuation-in-part of International Application PCT/CN2012/075706, filed May 18, 2012; which claims priority to Chinese Application No. 2011 10132218.9, filed May 20, 2011; both of which are incorporated by reference herein in their entirety, including any figures, tables, nucleic acid sequences, amino acid sequences, or drawings.

The Sequence Listing for this application is labeled "SeqList-10 Jun. 14-ST25.txt" which was created on Jun. 10, 2014 and is 105 KB. The entire content of the sequence listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention belongs to the field of biotechnology and relates to the treatment of diseases, especially the treatment of FGF overexpression-related diseases. Particularly, the present invention relates to FGFR-Fc fusion proteins and the use thereof in the treatment of angiogenesis regulation-related diseases. More particularly, the present invention relates to isolated soluble FGFR-Fc fusion proteins and their applications in manufacture of the medicament for the treatment of angiogenesis regulation-related diseases.

BACKGROUND OF THE INVENTION

Angiogenesis is one of the primary factors resulting in the growth and metastasis of malignant tumors [1]. The process of angiogenesis is regulated by many factors, among which some factors promote angiogenesis, while some factors inhibit angiogenesis, and as a result, the regulation of angiogenesis is a very complicated dynamic process [2]. Anti-angiogenesis treatment is intended to control the growth of a tumor by blocking angiogenic stimulating factors or preventing angiogenesis in the tumor using angiogenesis inhibitors. At present, a large amount of angiogenic stimulating factors are known, such as, for example, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF) etc. which may stimulate the division and differentiation of vascular endothelial cells and the morphogenesis of blood vessels. Among these factors mentioned above, it is now known that VEGF is the most angiogenesis-specific and the most effective growth factor [3, 4].

In a hypoxic environment inside tumor tissue, VEGFs are secreted by the tumor cells, which induce the division and migration of vascular endothelial cells, resulting in the establishment of a tumor vascular network. It has been demonstrated that the inhibition of VEGF may prevent angiogenesis, and further inhibit the growth of tumor. For this reason, VEGF and its receptors are important targets for anti-angiogenesis medicaments.

At present, anti-angiogenesis medicaments demonstrated in clinical trials to have efficacy include Bevacizumab (under the trade name of Avastin), which is able to block VEGF directly and inhibit the tumor angiogenesis. Bevacizumab was approved for marketing by the FDA in 2004, and as a first-line drug for rectal cancer, it is the first marketing-approved drug that plays a role in anticarcinogenesis by inhibiting angiogenesis. Avastin is a humanized anti-VEGF monoclonal antibody, which is produced by Genentech. In a large-scale Phase III clinical trial, the combined therapy by Avastin

and chemotherapy may significantly extend the survival time of the patients suffered from many kinds of cancers, including rectal cancer, lung cancer, breast cancer and renal cancer. [5, 6] The clinical success of Avastin is a landmark, demonstrating that the anti-angiogenesis treatment using tumor vascular system as the target is a clinically effective measure and provide a new path for the tumor treatment.

Besides Avastin, several drugs for anti-VEGF signaling are also in the late phase of human clinical trial and are expected for clinical application in the next several years. Among others, Aflibercept (also called as VEGF-Trap), developed by the Regeneron and Sanofi-Aventis, is now in Phase III clinical trial [7]. An anti-VEGF receptor II (VEGFR2) monoclonal antibody drug IMC-1121B (Imclone) is also in Phase III clinical trial [8]. The amino acid sequence of VEGFR2 is provided in SEQ ID NO: 3 and the nucleotide sequence of VEGFR2 mRNA is provided in SEQ ID NO: 6.

Great progress has been achieved in the clinical treatment of tumor using anti-VEGF medicament, however, it has also been shown by the clinical trial that the anti-VEGF treatment are also considerably limited. From the point of the effect of tumor treatment, Avastin may extend the half survival time of the colon cancer patient for about 3-4 months [9, 10], and extend the half survival time of the breast cancer patient for about 7-8 months [11], and thus, Avastin cannot effectively inhibit the growth of tumor blood vessel over the long term.

The primary causes resulting in the failure of anti-VEGF treatment or the appearance of resistance may depend on the regulation of tumor angiogenesis by a plurality of factors. Although VEGF plays an important role in angiogenesis, it is not the only angiogenesis stimulating factor. Meanwhile, owing to the heterogeneity of tumor cells, the complexity of tumor microenvironment and the compensatory response mechanism of body, when the activity of VEGF is inhibited for a long period of time, other angiogenesis stimulating factors would be expressed [12], and thus the growth of tumor blood vessel is no longer dependent on VEGF signaling path.

The variation of angiogenesis factors expressed by the tumor was studied during anti-VEGFR2 treatment for pancreatic tumor by Prof Hanahan's group (University of California, San Francisco, US), indicating that the expression of several genes changed during anti-VEGF treatment, in which the expression of FGF-2 significantly increased. It has been shown that the expression of FGF, especially FGF-2, increased significantly in the tumor resistant to anti-VEGF treatment so that angiogenesis was activated again and the tumor repopulation was inhibited after blocking FGF signal pathway [13]. It may be seen that the over-expression of FGF-2 is closely related to the ability of tumor to escape from anti-VEGF treatment.

Fibroblast growth factor (FGF) is a growth factor family for heparin-binding, and there are 22 family members (FGF 1-14, 16-23) in mammals. FGF plays an important role in many biological functions, for example, cell proliferation, differentiation, migration, angiogenesis and tumorigenesis. Fibroblast growth factor receptor (FGFR) is the receptor that binds the family members of fibroblast growth factor. FGF may bind FGFR and activate the downstream signal pathway, which plays an important role in a physiological and pathological process, such as embryogenesis, development, vasculogenesis, vasodilatation, neuroregulation, ischemia protection, wound healing and tumorigenesis. [14, 15] It has been demonstrated that overexpression of FGF/FGFR in vivo is closely related to many diseases including tumors (such as fibroma, neuroglioma, melanoma, prostate carcinoma, lymphomata, leukaemia, urinary, and system cancer), skeletal system diseases (dwarfism, craniosynostosis, achondroplasia

sia, and acanthosis nigricans) and renal failure. It has been reported that increased expression level of FGF and its receptor may directly promote the survival and proliferation of tumor cells, and the survival of hepatic carcinoma cells is significantly reduced by down-regulation of FGF by siRNA [22].

At present, few researches focus on the development of new anti-angiogenesis medicament using FGF and its receptor as the target in clinical trials. For example, FP-1039, a fusion protein composed of whole extracellular domain of human FGFR1 and human IgG1 Fc fragment, is developed by a US company Five Prime and now in volunteer recruitment stage of Phase I clinical trial. However, it has been suggested by researches of Wang and Olsen that the first Ig-like domain of the extracellular domain of human FGFR1 and the linking fragment between the first and the second Ig-like domain of the extracellular domain of human FGFR1 may inhibit binding of FGFR1 and FGF [20, 21].

The tertiary structure of a protein is closely related to its biological function. The FGF binding capacity is directly influenced differences among the conformations of each Ig-like domain of the extracellular domain of FGFR and the linking fragment. Different fusion proteins, composed of the FGFR extracellular domain fragments of various lengths and IgG Fc, are constructed by means of genetic engineering to obtain fusion proteins with different conformations, so that the fusion protein with high efficiency of FGF binding and biological activity can be screened.

There are four FGFR genes in mammals: fgfr1-fgfr4. Fibroblast growth factor receptor is composed of the extracellular domain, transmembrane domain and intracellular domain. There are many members in FGFR family, which have similar extracellular domain but vary in the ligand binding property and kinase domain. Their extracellular domains include three immunoglobulin-like (Ig-like) domains: the first Ig-like domain, the second Ig-like domain and the third Ig-like domain, and there is a sequence between the first and the second Ig-like domain, which is referred as the intermediate functional sequence of the Ig-like domain of FGFR (IFS for short herein) in this specification. The intermediate functional sequence may comprise one acidic amino acid segment, which is referred as acidic box (AB).

BRIEF SUMMARY

The present invention provides isolated soluble fusion proteins of fibroblast growth factor receptor (FGFR), which comprise: a part derived from an intermediate functional sequence (also referred to herein as IFS) of an Ig-like domain of FGFR, a second Ig-like domain (also referred to herein as D2) of FGFR, a third Ig-like domain (also referred to herein as D3) of FGFR and an immunoglobulin Fc region.

In certain embodiments, the part derived from IFS contains no acidic box. In other embodiments, the IFS portion has the amino acid sequence of position 134 to position 162, position 145 to position 162, or position 151 to position 162 of SEQ ID NO: 1, or has an amino acid sequence sharing at least 70% identity with the amino acid sequence of position 134 to position 162, position 145 to position 162 or position 151 to position 162 of SEQ ID NO: 1.

The present invention further relates to a fusion protein, which comprises or consists of: the first Ig-like domain (also referred to herein as D1) of FGFR or a moiety thereof, a part derived from the intermediate functional sequence region of the Ig-like domain of FGFR, the second Ig-like domain of FGFR, the third Ig-like domain of FGFR, and an immunoglobulin Fc region.

Preferably, the D1 domain or a moiety thereof possesses:

the amino acid sequence corresponding to position 40 to position 118 of SEQ ID NO: 1, or an amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with the sequence of position 40 to position 118 of SEQ ID NO: 1; or the amino acid sequence corresponding to position 77 to position 118 of SEQ ID NO: 1, or an amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with the amino acid sequence of position 77 to position 118 of SEQ ID NO: 1.

In one aspect, the present invention provides a fusion protein that comprises or consists of: the intermediate functional sequence region of the Ig-like domain of FGFR or a moiety thereof, the second Ig-like domain of FGFR, the third Ig-like domain of FGFR and immunoglobulin Fc region, wherein:

the second Ig-like domain of FGFR has the amino acid sequence corresponding to position 163 to position 247 of SEQ ID NO: 1, or an amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with the amino acid sequence of position 163 to position 247 of SEQ ID NO: 1; and/or

the third Ig-like domain of FGFR has the amino acid sequence corresponding to position 270 to position 359 of SEQ ID NO: 1, or an amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with the amino acid sequence of position 270 to position 359 of SEQ ID NO: 1.

The present invention further provides a fusion protein that comprises a region derived from the extracellular domain of FGFR and an immunoglobulin Fc region or composed thereof, wherein the region derived from the extracellular domain of FGFR:

(1) has the amino acid sequence indicated by positions 358-580 of SEQ ID NO: 9, positions 304-526 of SEQ ID NO: 10, positions 278-500 of SEQ ID NO: 11, positions 246-468 of SEQ ID NO: 12, positions 235-457 of SEQ ID NO: 13, positions 229-451 of SEQ ID NO: 14 or positions 224-446 of SEQ ID NO: 15, or the amino acid sequence encoded by the nucleotide sequence indicated by positions 1074-1740 of SEQ ID NO: 16, positions 912-1578 of SEQ ID NO: 17, positions 834-1500 of SEQ ID NO: 18, positions 738-1404 of SEQ ID NO: 19, positions 705-1371 of SEQ ID NO: 20, positions 687-1353 of SEQ ID NO: 21 and positions 672-1338 of SEQ ID NO: 22;

(2) comprises or consists of the amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with the amino acid sequence indicated by positions 358-580 of SEQ ID NO: 9, positions 304-526 of SEQ ID NO: 10, positions 278-500 of SEQ ID NO: 11, positions 246-468 of SEQ ID NO: 12, positions 235-457 of SEQ ID NO: 13, positions 229-451 of SEQ ID NO: 14 or positions 224-446 of SEQ ID NO: 15; or

(3) comprises or consists of the amino acid sequence encoded by a nucleotide sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with the nucleotide sequence indicated by positions 1074-1740 of SEQ ID NO: 16, positions 912-1578 of SEQ ID NO: 17, positions 834-1500 of SEQ ID NO: 18, positions 738-1404 of SEQ ID NO: 19, positions 705-1371 of SEQ ID NO: 20, positions 687-1353 of SEQ ID NO: 21 or positions 672-1338 of SEQ ID NO: 22.

The present invention further relates to a fusion protein, wherein the protein:

(1) comprises the amino acid sequence indicated by any one of SEQ ID NOs: 9-15, or an amino acid sequence encoded by the nucleotide sequence indicated by any one of SEQ ID NOs: 16-22;

(2) comprises or consists of the amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity, with the amino acid sequence indicated by any one of SEQ ID NOs: 9-15; or

(3) comprises or consists of the amino acid sequence encoded by the nucleotide sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity, with the nucleotide sequence indicated by any one of SEQ ID NOs: 16-22.

Preferably, in the fusion protein of the present invention, the immunoglobulin Fc region is human IgG1 Fc region, and more preferably, it comprises:

the amino acid sequence corresponding to SEQ ID NO: 7, or the amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity, with the amino acid sequence of SEQ ID NO: 7; or

the amino acid sequence encoded by the nucleotide sequence corresponding to SEQ ID NO: 8, or the amino acid sequence encoded by the nucleotide sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity, with the nucleotide sequence of SEQ ID NO: 8.

In one embodiment of the present invention, the immunoglobulin Fc region is located at the C-terminus of the fusion protein.

The present invention further provides a fusion protein precursor comprising a secretory signal peptide region, for example, VEGFR1 signal peptide region, and preferably, the secretory signal peptide region has the amino acid sequence of position 1 to position 26 of SEQ ID NO: 2 or the amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 23. Preferably, the signal peptide region is located at the N-terminus of the precursor. The nucleotide sequence of VEGFR1 mRNA is provided in SEQ ID NO: 5.

In another aspect of the present invention, an isolated nucleic acid molecule that encodes the fusion protein or the precursor of the fusion protein of the present invention is provided. Preferably, the nucleic acid molecule comprises the nucleotide sequence indicated by any one of SEQ ID NOs: 16-22.

The present invention provides a fusion protein that sequentially comprises from the N-terminus to the C-terminus: portions derived from IFR, D2, D3 and immunoglobulin Fc region.

The domains and/or regions involved in the fusion protein of the present invention can be linked directly and/or by a linker. In one embodiment, the region derived from the extracellular domain of FGFR and immunoglobulin Fc region are linked directly. In another embodiment, the region derived from the extracellular domain of FGFR and immunoglobulin Fc region are linked by a linker.

In one aspect, the fusion protein of the present invention inhibits angiogenesis. In another aspect, the fusion protein of the present invention binds FGF, preferably FGF2, in vivo and/or in vitro. In another aspect, the fusion protein of the present invention inhibits tumor cells directly.

The present invention further relates to an FGFR-Fc fusion protein that comprises a portion derived from the extracellular domain of FGFR and a portion derived from immunoglobulin Fc region. Particularly, the portion derived from the extracellular domain of FGFR is derived from the extracellular

lar domain of FGFR1. Preferably, the immunoglobulin Fc region is a human immunoglobulin Fc region, for example, a human IgG1 Fc region. In one aspect of the present invention, the FGFR-Fc fusion protein of the present invention has the capacity of binding and/or antagonizing FGF, and thus, inhibit angiogenesis.

In the FGFR-Fc fusion protein of the present invention, the portion derived from the extracellular domain of FGFR may comprise one or more selected from the group consisting of: D1 domain or a moiety thereof, the portion derived from IFS, D2 domain or a moiety thereof and D3 domain or a moiety thereof.

In one embodiment, the part derived from the extracellular domain of FGFR may comprise D1 or a moiety thereof, the part derived from IFS, D2 domain and D3 domain.

In another embodiment, the part derived from the extracellular domain of FGFR may comprise the part derived from IFS, D2 domain and D3 domain, and preferably, the part derived from IFS has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162, or position 151 to position 162 of SEQ ID NO: 1.

In some embodiments, the FGFR-Fc fusion protein of the present invention contains no D1 or a moiety thereof. In some other embodiments, the FGFR-Fc fusion protein of the present invention contains no part from IFS other than the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162, or position 151 to position 162 of SEQ ID NO: 1.

In some embodiments of the present invention, the order from the N-terminus to the C-terminus of each region and/or each domain involved in the FGFR-Fc fusion protein may be any order. In some other embodiments, the order can be as shown in FIG. 1. In some other embodiments, the order may be different from the order shown in FIG. 1.

In some embodiments, the FGFR-Fc fusion protein of the present invention further comprises one or more intrachain disulfide bonds, and preferably, comprises one or more intrachain disulfide bonds in the Ig-like domain.

In one aspect of the present invention, the FGFR-Fc fusion protein can be produced by expression of the nucleic acid comprising the nucleotide sequence indicated by any one of SEQ ID NOs: 16-22 in a mammalian cell line. The mammalian cell line can be, for example, a CHO cell line.

In another aspect of the present invention, a vector comprising the nucleic acid molecule of the present invention is provided.

In another aspect of the present invention, cells, such as CHO cells, transfected by the vector are provided.

In another embodiment of the present invention, a pharmaceutical composition, which comprises the fusion protein, the nucleic acid molecule, the vector, or the cells of the present invention, as well as a pharmaceutically acceptable carrier, is also provided.

In another aspect, the present invention provides a method for producing the angiogenesis-inhibitory fusion protein, which is carried out by expressing the fusion protein of the present invention in prokaryotic cells or eukaryotic cells, especially, in mammalian cell lines.

The present invention further provides a method for producing the angiogenesis-inhibitory fusion protein, which is carried out by expressing the nucleic acid molecule of the present invention in a mammalian cell line. The mammalian cell line can be, for example, a CHO cell line.

In another aspect of the present invention, a method for inhibition of angiogenesis is provided, which comprises administering, to a subject in need thereof, an angiogenesis-inhibiting effective amount of the FGFR-Fc fusion protein,

the nucleic acid molecule encoding the protein, the vector comprising the nucleic acid molecule and/or a pharmaceutical composition comprising any one of these materials. Preferably, the method is carried out in a mammal.

In another aspect of the present invention, a method for the treatment and/or prevention of a tumor in a mammal is provided. This method comprises administering, to a need of such treatment, a therapeutically or preventively effective amount of the FGFR-Fc fusion protein, the nucleic acid molecule encoding the protein, the vector comprising the nucleic acid molecule, and/or a pharmaceutical composition comprising any one of these materials. Preferably, the tumor is a solid tumor.

In another aspect, the present invention provides a method for the treatment or prevention of ophthalmic angiogenesis-related diseases in mammals. This method comprises administering, to a subject in need of such treatment or prevention, a therapeutically or preventively effective amount of the FGFR-Fc fusion protein, the nucleic acid molecule encoding the protein, the vector comprising the nucleic acid molecule, and/or a pharmaceutical composition comprising any one of these materials. Preferably, the ophthalmic angiogenesis-related disease is age-related macular degeneration.

The present invention further relates to use of the FGFR-Fc fusion protein, the nucleic acid molecule encoding the protein, the vector comprising the nucleic acid molecule, and/or a pharmaceutical composition comprising any one mentioned above according to the present invention in the manufacture of a medicament for inhibiting angiogenesis.

Furthermore, the present invention further relates to use of the FGFR-Fc fusion protein, the nucleic acid molecule encoding the protein, the vector comprising the nucleic acid molecule, and/or a pharmaceutical composition comprising any one mentioned above according to the present invention in manufacture of a medicament for the treatment or prevention of angiogenesis-related diseases, and preferably, the angiogenesis-related disease is a tumor or ophthalmic angiogenesis-related disease.

In the disclosure, only some specific embodiments claimed for protection are illustrated by way of example, in which the technical features described in one or more technical proposals can be combined with any one or more technical proposals, and these technical proposals obtained by combination are also within the scope of this application, as if these technical proposals obtained by combination were already specifically described in the disclosure.

It should be understood that the description below is only illustrated by way of example for the technical solutions claimed for protection by the present invention, and not regarded as any limitation on these technical solutions. The protection scope of the present invention shall be defined by the claims as appended.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a structural representation of a FGFR1-Fc fusion protein. The FGFR1-Fc fusion protein is represented by a solid line, and a deleted amino acid is represented by a dashed line; the antibody-like domain is represented by a circle; different antibody-like domains are represented by numbers 1-3; a disulfide bond is represented by s; human IgG1 Fc is

represented by a grey box; VEGFR1 signal peptide is represented by SP; the acidic box sequence is represented by a box with the letters AB.

FIG. 2 shows a comparison of FGF-2 binding among various FGFR1-Fc fusion proteins. Binding of heparin (100 ng/mL) containing FGF-2 (50 ng/mL) or FGF-2 (50 ng/mL) alone to each FGFR1-Fc fusion protein (20 ng/mL) is detected by ELISA.

FIG. 3 shows SDS-PAGE of 26# FGFR1-Fc fusion protein. FIG. 4 shows the binding of FGF-2 to a gradient concentration of 26# FGFR1-Fc fusion protein.

FIG. 5 shows the affinity between 26# FGFR1-Fc fusion protein and FGF-2.

FIG. 6 shows the effect of 26# FGFR1-Fc fusion protein on the HUVEC cell division induced by FGF-2.

FIG. 7 shows the anti-tumor efficacy of FGFR-Fc in renal carcinoma model

FIG. 8 shows the anti-tumor efficacy of FGFR-Fc in lung carcinoma model

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 is an amino acid sequence of human FGFR1.

SEQ ID NO:2 is an amino acid sequence of human VEGFR1.

SEQ ID NO:3 is an amino acid sequence of human VEGFR2.

SEQ ID NO:4 is a nucleotide sequence of human FGFR1.

SEQ ID NO:5 is a nucleotide sequence of human VEGFR1.

SEQ ID NO:6 is a nucleotide sequence of human VEGFR2.

SEQ ID NO:7 is an amino acid seq of human IgG Fc.

SEQ ID NO:8 is a DNA seq of human IgG Fc.

SEQ ID NO:9 is a #19 fusion protein.

SEQ ID NO:10 is a #13 fusion protein.

SEQ ID NO:11 is a #22 fusion protein.

SEQ ID NO:12 is a #23 fusion protein.

SEQ ID NO:13 is a #26 fusion protein.

SEQ ID NO:14 is a #29 fusion protein.

SEQ ID NO:15 is a #8 fusion protein.

SEQ ID NO:16 is a DNA seq of #19 fusion protein.

SEQ ID NO:17 is a DNA seq of #13 fusion protein.

SEQ ID NO:18 is a DNA seq of #22 fusion protein.

SEQ ID NO:19 is a DNA seq of #23 fusion protein.

SEQ ID NO:20 is a DNA seq of #26 fusion protein.

SEQ ID NO:21 is a DNA seq of #29 fusion protein.

SEQ ID NO: 22 is a DNA seq of #8 fusion protein.

SEQ ID NO:23 is a DNA seq of VEGFR1 signal peptide.

SEQ ID NO:24 is a forward primer DNA seq of #19 fusion protein.

SEQ ID NO:25 is a forward primer of DNA seq of #13 fusion protein.

SEQ ID NO:26 is a forward primer DNA seq of #22 fusion protein.

SEQ ID NO:27 is a forward primer DNA seq of #23 fusion protein.

SEQ ID NO:28 is a forward primer DNA seq of #26 fusion protein.

SEQ ID NO:29 is a forward primer DNA seq of #29 fusion protein.

SEQ ID NO:30 is a forward primer DNA seq of #8 fusion protein.

SEQ ID NO:31 is a reverse primer DNA seq of FGFR1.

SEQ ID NO: 32 is a forward primer DNA seq of human IgG Fc.

SEQ ID NO:33 is a reverse primer DNA seq of human IgG Fc.

DETAILED DESCRIPTION

Definitions

Unless otherwise defined, all scientific terms used herein have the same meaning as commonly understood by those skilled in the art. With regard to the definitions and terms in the art, reference may be made to Current Protocols in Molecular Biology (Ausubel) by the skilled one. Standard three- and/or one-letter code used for expressing one of 20 common L-amino acids in the art are adopted as the abbreviation of amino acid residues.

Although the number ranges and approximate parameter values are given in a broad range in the present invention, all numbers in the specific examples are described as precise as possible. However, certain errors can exist in any numerical values, which may result from, for example, the standard deviation during the measurement. Additionally, all ranges disclosed herein encompass any and all possible subranges contained therein. For example, it should be understood that the range “from 1 to 10” as described herein encompasses any and all possible subranges between the minimum 1 and the maximum 10 (including the endpoints). Additionally, it should be understood that any reference referred as “incorporated herein” is incorporated in its entirety.

Additionally, it should be noted that unless otherwise clearly and explicitly stated, the singular form includes the plural referent, as used in the present invention. The term “or” and the term “and/or” are used interchangeably, unless otherwise clearly indicated in the context.

As used herein, the term “Fc”, “Fc region”, “Fc fragment” or “immunoglobulin Fc region” refers to the crystallizable fragment of immunoglobulin, and in the present invention, said Fc region is preferably the human IgG1 Fc region.

The term “Fc fusion protein” refers to the antibody-like molecule that incorporates the binding specificity of a heterologous protein and the effector function of a constant region of an immunoglobulin. In terms of the molecular structure, a Fc fusion protein comprises the amino acid sequence having the required binding specificity and the sequence of a constant region of an immunoglobulin. A Fc fusion protein molecule generally comprises a binding site of a receptor or a ligand. The sequence of immunoglobulin constant region may be derived from any immunoglobulin, for example, IgG-1, IgG-2, IgG-3 or IgG-4 subtype, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

The term “soluble” protein as used herein refers to a protein that may be dissolved in an aqueous solution at a biologically relevant temperature, pH level and osmotic pressure. The “soluble fusion protein” as used herein is intended to mean that the fusion protein does not contain a transmembrane region or an intracellular region.

As used herein, the term “isolated” refers to a substance and/or entity that: (1) is isolated from at least some components which is present when initially produced (in natural environment and/or in an experiment device) and related thereto and/or (2) is produced, prepared and/or manufactured artificially. The isolated substance and/or entity may be isolated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98%, about 99%, substantially 100% or 100% other components related to it initially.

The terms “part,” “fragment,” or “portion” interchangeably refer to a part of polypeptide, nucleic acid or other molecular constructs.

The term “Ig-like domain” as used herein refers to immunoglobulin-like domain, which may be found in a plurality of protein families and involved in many biological functions, including cell-cell recognition, cell surface receptor, immune function and the like.

Fibroblast growth factor (FGF) is a heparin-binding growth factor family that has 22 family members in mammals (FGF 1-14, 16-23). FGF is involved in many important biological functions, such as cell multiplication, differentiation, migration, angiogenesis and tumorigenesis. FGF exerts many biological functions by binding and activating the cell surface FGF receptor (FGFR). (See, for example, Eswarakumar et al. *Cytokine Growth Factor Rev.* 16: 139-149, 2005).

Fibroblast growth factor receptor (FGFR) is the receptor that binds the family members of fibroblast growth factor. A part of fibroblast growth factor receptor is involved in the disease process. In mammals, there are 4 FGFR genes: fgfr1-fgfr4. The fibroblast growth factor receptor is composed of an extracellular domain, transmembrane domain, and intracellular domain. The members in FGFR family can differ from each other in the term of ligand binding properties and kinase domains. However, the extracellular domains thereof are similar. There are three immunoglobulin-like (Ig-like) domains contained in their extracellular domains: the first Ig-like domain, the second Ig-like domain and the third Ig-like domain, and there is also a sequence contained between the first and the second Ig-like domain. The sequence contained between the first and the second Ig-like domain is referred to herein as the intermediate functional sequence region of the Ig-like domain of FGFR. Said intermediate regulation sequence comprises a region of acidic amino acids, referred as the acidic box (AB).

As used herein, the term “the first Ig-like domain of FGFR” or “the first Ig-like domain” refers to the first Ig-like domain in the protein FGFR from the N-terminus, which has, for example, the amino acid sequence corresponding to position 40 to position 118 of SEQ ID NO: 1. Similarly, the term “the second Ig-like domain of FGFR” or “the second Ig-like domain” refers to the second Ig-like domain in the protein FGFR from the N-terminus, which has, for example, the amino acid sequence corresponding to position 163 to position 247 of SEQ ID NO: 1; the term “the third Ig-like domain of FGFR” or “the third Ig-like domain” refers to the first Ig-like domain in the protein FGFR from the N-terminus, which has, for example, the amino acid sequence corresponding to position 270 to position 359 of SEQ ID NO: 1.

Preferably, the FGFR is FGFR1, and the first Ig-like domain of FGFR is the first Ig-like domain of FGFR1, and the second Ig-like domain of FGFR is the second Ig-like domain of FGFR1, and the third Ig-like domain of FGFR is the third Ig-like domain of FGFR1.

A part of sequence of hFGFR1 is given as follows, in which each Ig-like domain is shown in shaded area sequentially, see GenBank AAH15035.1

MWSWKCLFLWAVLVTATLCTARPSPTLPEQAQPGWAPVEVESFLVHPGDLQLRCRLR
DDVQSINWLRDGVQLAESNRTRITGEEVEVQDSVPADSGLYACVTSSPSGSDTTYFSVN
VSDALPSSSEDDDDDDSSSEKETDNTKPNPVAPYWTSPKMEKKLHAYPAKTIKPKK
CFSSGIGQTPNPTLRLKNGKEFKFDRHICGYIVRYATWSIINDSVPSDKKNTTCIVEN
EYGSINHTYQLDQVYERSPHRPILOAGLPANKTVLGSNVEFMCKVYEDPOPHIQWLHH
EVNGSKIGDPIGDNLPIYQILKTAGVNTTKEMEVLHKNVSPEDAGEYTCACNIGIS
HHSAWITVLEALEER

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The amino acid sequence of FGFR1 may be found in SEQ ID NO: 1, and its encoding nucleotide sequence may be found in SEQ ID NO: 4.

As used herein, the term “the intermediate functional sequence region of the Ig-like domain of FGFR” or “the intermediate functional sequence of the Ig-like domain of FGFR” or “IFS” refers to the sequence between the first Ig-like domain and the second Ig-like domain in the protein FGFR, and preferably, IFS sequence has the amino acid sequence corresponding to position 118 to position 162 of SEQ ID NO: 1.

Unexpectedly, in accordance with the present invention, it has been found that there is a significant effect of the intermediate functional sequence region on the function of the Ig-like domain. In some embodiments of the present invention, the part derived from the intermediate functional sequence region contains no acidic box. More preferably, the part derived from IFS has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162, or position 151 to position 162 of SEQ ID NO: 1.

The protein FGFR is preferably FGFR1 (SEQ ID NO: 1), especially the protein FGFR1. The amino acid sequence of the human FGFR1 is shown in SEQ ID NO: 1, and its cDNA sequence is shown in SEQ ID NO: 4.

The term “FGFR” as used herein refers to fibroblast growth factor receptor, which may be FGFR1, FGFR2, FGFR3 and/or FGFR4. Preferably, the FGFR of the present invention is FGFR1, more preferably, human FGFR1.

As used herein, the term “degenerate variant” means that the degenerate variant comprises a degenerate change at the third position of the amino acid codon so that degenerate variants encode the same amino acid, for example the wobble position of a triplet code comprising one or more changed variants (also referred as synonymous variant).

As used herein, the term “subject” refers to mammals, such as humans. It also includes other animals, including domesticated animals (such as dogs and cats), livestock (such as cattle, sheep, pigs and horses) or experimental animals (such as monkeys, rats, mice, rabbits and guinea pigs).

As used herein, the term “percentage identity,” “homology,” or “identity” referred to the sequence identity between two amino acid sequences or nucleic acid sequences. The percentage identity may be determined by alignment between two sequences, and the percentage identity refers to the amount of the same residue (i.e., amino acid or nucleotide) at the same position in the aligned sequences. Sequence alignment and comparison may be performed using standard algorithms in the art (for example Smith and Waterman, 1981, *Adv. Appl. Math.* 2: 482; Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443; Pearson and Lipman, 1988, *Proc. Natl.*

Acad. Sci., USA, 85: 2444) or by the computerized versions of these algorithms (Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive, Madison, Wis.). Computerized versions that are publicly available include BLAST and FASTA. Additionally, ENTREZ available through National Institutes of Health (Bethesda Md.) may be used for sequence alignment. When BLAST and GAP-BLAST are used, default parameters for each program (for example, BLASTN, available on the website of National Center for Biotechnology Information) may be used. In one embodiment, the percentage identity between two sequences may be determined using GCG with a gap-weight of 1 so that the giving weight of each amino acid gap seems as if it is a single amino acid mismatch between two sequences. Alternatively, ALIGN (version 2.0), which is a part of GCG (Accelrys, San Diego, Calif.) Sequence Alignment Software Package, may be used.

As used herein, the term “hybridization” refers to the process by which a stable double-stranded polynucleotide is formed by non-covalent bonding between two single stranded polynucleotides. The term “hybridization” also may refer to triple-stranded hybridization. The double stranded polynucleotide (generally) produced is the “hybrid” or “duplex”. “The condition for hybridization” generally includes a salt concentration lower than about 1 M, and more generally, lower than about 500 mM, and lower than about 200 mM. The hybridization temperature may be as low as 5° C., but it usually higher than about 22° C., and more usually higher than about 30° C., and preferably higher than about 37° C. Hybridization is usually carried out under strict conditions (i.e., the conditions under which the probe will hybridize to its target sequence). Strict hybridization conditions are dependent on the sequence and will be varied under different conditions. Higher hybridization temperature will be probably required by longer segments for specific hybridization. Since the hybridization stringency may be influenced by other factors (including base composition and length of the complementary strand, the presence of organic solvent and the degree of base mismatch), the combination of parameters is more important than the absolute value of any single parameter. Generally, the strict condition is selected as 5° C. lower than the T_m of the sequence under certain ionic strength and pH. Exemplary strict conditions include pH 7.0 to 8.3, sodium ion (or other salts) concentration of at least 0.01 M to no more than 1 M and temperature of at least 25° C. For strict conditions, see, for 2nd example Sambrook, Fritsche and Maniatis. “Molecular Cloning A laboratory Manual”, edition, Cold Spring Harbor Press (1989) and Anderson “Nucleic Acid Hybridization”, 1st edition, BIOS Scientific Publishers Limited (1999), which are incorporated herein by reference for all purposes mentioned above.

As used herein, the terms "linker," "peptide linker," "linking sequence," and "linker sequence" refer to a short amino acid sequence by which individual domain and/or region involved in the present fusion protein are linked together. The length of the short amino acid sequence is generally 1-20 amino acids, and preferably, 2-10 amino acids.

As used herein, the term of "the amino acid sequence corresponding to SEQ ID NO: N" in a fusion protein or part or domain means that the fusion protein or part or domain has the amino acid sequence substantially as indicated by SEQ ID NO: N, and preferably, containing no more than 1, 2, 3, 4, 5, 10 or 20 substitutions, additions, and/or deletions of amino acids, and preferably, the fusion protein or part or domain shares at least 70%, 80%, 90%, 93%, 95%, 97%, 98% or 99% identity with the amino acid sequence of SEQ ID NO: N, and more preferably, said fusion protein or part or domain has the amino acid sequence as indicated by SEQ ID NO: N.

As used herein, the term "FGFR-Fc fusion protein" refers to a fusion protein that comprises the part derived from the extracellular domain of FGFR and the part derived from the immunoglobulin Fc region, wherein the part derived from the extracellular domain of FGFR may: (1) comprise the amino acid sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity, with the amino acid sequence indicated by any one of SEQ ID NOs: 9-15 or composed thereof; (2) comprise the amino acid sequence encoded by the nucleotide sequence sharing at least 70% identity, preferably at least 80%, 90%, 93%, 95%, 97%, 98% or 99% identity, with the nucleotide sequence indicated by any one of SEQ ID NOs: 16-22 or composed thereof; or (3) possess the amino acid sequence indicated by any one of SEQ ID NOs: 9-15, or the amino acid sequence encoded by the nucleotide sequence indicated by any one of SEQ ID NOs: 16-22.

In some preferable embodiments, the FGFR-Fc fusion protein may be encoded by a nucleic acid, in which the nucleotide sequence encoding the part derived from the extracellular domain of FGFR comprises the sequence of which a complementary sequence is hybridized with the nucleotide sequence as indicated by any one of SEQ ID NOs: 16-22 under stringent conditions, or comprises a degenerative variant of the nucleotide sequence as indicated by any one of SEQ ID NOs: 16-22. In some preferable embodiments, the nucleotide sequence encoding the immunoglobulin Fc region comprises the sequence of which a complementary sequence is hybridized with the nucleotide sequence indicated by SEQ ID NO: 8 under stringent conditions, or comprises a degenerative variant of the nucleotide sequence indicated by SEQ ID NO: 8.

In other preferable embodiments, the FGFR-Fc fusion protein includes the FGFR-Fc fusion protein variant. In one embodiment, the variant includes the variant that contains no more than 2, 3, 4, 5 or 10 substitutions, additions or deletions of amino acid in the part derived from IFS corresponding to the amino acid sequence indicated by position 134 to position 162, position 145 to position 162, or position 151 to position 162 of SEQ ID NO: 1, and preferably, the variant retains the angiogenesis-inhibitory capacity. In another embodiment, the variant contains no more than 2, 3, 4, 5, 10 or 20 substitutions, additions and/or deletions of amino acids in the D2 domain corresponding to the amino acid sequence indicated by position 163 to position 247 of SEQ ID NO: 1, and preferably, the variant retains the angiogenesis-inhibitory capacity. In another embodiment, the variant contains no more than 2, 3, 4, 5, 10 or 20 substitutions, additions and/or deletions of amino acid in D3 domain corresponding to the amino acid sequence indicated by position 270 to position 359 of SEQ ID

NO: 1, and preferably, the variant retains the angiogenesis-inhibitory capacity. In another embodiment, the substitution, addition, or deletion is located at the linker or the linking part.

In addition to the naturally occurring modifications in the part derived from the extracellular domain of FGFR and the part derived from immunoglobulin Fc region, other post-translational modifications may also be comprised in the FGFR-Fc fusion protein. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, esterification and acylation. As a result, non-amino acid components may exist in the modified FGFR-Fc fusion protein. These components may be, for example, polyethylene glycol, lipid, polysaccharide or monosaccharide, or phosphoric acid. The effect of such non-amino acid components on the function of the FGFR-Fc fusion protein may be tested as described for other FGFR-Fc fusion protein variants herein. When the FGFR-Fc fusion protein is produced in a cell, post-translational processing is also possibly important for correct folding and/or protein function. Special cell machines and unique mechanisms exist in different cells (for example CHO, HeLa, MDCK, 293, WI38, NIH-3T3 or HEK293) for these post-translational activities, and different cells may be selected by the skilled artisan to improve modification and processing of FGFR-Fc fusion protein.

The fusion protein as described herein may be produced by any method known in the art. For example, it may be produced by chemical synthesis or from nucleic acid expression. The peptides used in the present invention may be easily prepared according to the established standard liquid, or preferably, solid phase peptide synthesis method known in the art (see, for example J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford, Ill. (1984), in M. Bodanzsky, and A. Bodanzsky, The Practice of Peptide Synthesis, Springer Verlag, New York (1984)). The fusion protein may be produced by the techniques known in the art so that one or more intramolecular crosslinkings may be formed between the cysteine residues located in the polypeptide sequence expected to be comprised in the protein (see, for example U.S. Pat. No. 5,478,925). In addition, general modifications may be performed to the protein described herein by adding, for example, cysteine or biotin to the C-terminus or N-terminus of the protein.

As used herein, "therapeutically effective amount" or "effective amount" refers to a dosage that is sufficient to provide a benefit to the subject to whom it is administered. The administered dosage, the rate and the time course of administration are dependent on the condition of the patient and the severity of the disease. Finally, the physician is responsible for the prescription (for example decision on the dosage etc.) and will make a decision for the treatment, usually by considering the disease treated, individual condition of the patient, position of delivery, the method for administration and other factors known to the physician.

A series of isolated soluble FGFR-Fc fusion proteins have been constructed according to the present invention, which may bind FGF and effectively inhibit the cell division induced by FGF. The fusion protein preferably comprises: the part derived from IFS, D2, D3 and immunoglobulin Fc region.

Unexpectedly, it has also been found that the binding of FGF by the fusion protein is significantly influenced by the length of the part derived from IFS. Preferably, the part derived from IFS comprises no acidic box, and more preferably, it has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162, or position 151 to position 162 of SEQ ID NO: 1. In some preferable embodiments, the part derived from IFS comprises the fusion

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protein corresponding to the amino acid sequence indicated by position 145 to position 162 of SEQ ID NO: 1, which has extremely high FGF affinity and potential to effectively inhibit cell division induced by FGF.

In some embodiments of the present invention, a soluble FGFR-Fc fusion protein is provided, which comprises: D1, a part derived from IFS, D2, D3 and an immunoglobulin Fc region. Preferably, the part derived from IFS comprises no acidic box, and more preferably, it has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162, or position 151 to position 162 of SEQ ID NO: 1.

In some other embodiments of the present invention, a soluble FGFR-Fc fusion protein is provided, which comprises: a part of D1, a part derived from IFS, D2, D3 and an immunoglobulin Fc region. Preferably, the part derived from IFS comprises no acidic box, and more preferably, it has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162, or position 151 to position 162 of SEQ ID NO: 1.

In some other embodiments of the present invention, a soluble FGFR-Fc fusion protein is provided, which comprises: a part derived from IFS, D2, D3 and an immunoglobulin Fc region. Preferably, the part derived from IFS comprises no acidic box, and more preferably, it has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162, or position 151 to position 162 of SEQ ID NO: 1.

In some other embodiments of the present invention, a soluble FGFR-Fc fusion protein is provided, which is sequentially composed of, from the N-terminus to the C-terminus, a part derived from IFS, D2, D3 and an immunoglobulin Fc region. Preferably, the part derived from IFS comprises no acidic box, and more preferably, it has the amino acid sequence corresponding to position 134 to position 162, position 145 to position 162, or position 151 to position 162 of SEQ ID NO: 1.

In some other embodiments of the present invention, an FGFR-Fc fusion protein is provided, which can inhibit tumor cells directly or indirectly. Preferably, the FGFR-Fc fusion protein of the present invention inhibits tumor cells directly. More preferably, the growth of tumor cells is inhibited by the FGFR-Fc fusion protein of the present invention by at least 10%, 20%, 30%, 40%, 50%, 80%, 90% or 95%. The tumor cells may be any tumor cells, for example, leukemia, lung cancer, liver cancer, head and neck cancer, stomach cancer, bladder cancer, or carcinoma of uterine or cervix etc. Preferably, the inhibition is achieved by direct binding to tumor cells.

In some embodiments, the present invention includes use of (i) a FGFR-Fc fusion protein, or (ii) a polynucleotide encoding such fusion protein, in the preparation of the compositions or medicaments for the treatment of diseases mediated by, or related to, angiogenesis. For example, in one embodiment, the present invention provides use of (i) FGFR-Fc fusion protein, or (ii) a polynucleotide encoding such fusion protein in the preparation of a medicament as an angiogenesis inhibitor.

In some embodiments, the FGFR-Fc fusion protein according to the present invention may be produced by the expression of the nucleotide sequence as indicated by any one of SEQ ID NOS: 16-22 in a mammalian cell line. The mammalian cell line can be, for example, a CHO cell line.

Additionally, in the present invention, the FGFR-Fc fusion protein as described below is provided, in which a part derived from the extracellular domain of FGFR may be fused with the immunoglobulin Fc region with or without a linker.

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In some other embodiments, the present invention includes the isolated nucleic acid molecules encoding the FGFR-Fc fusion protein, and the present invention also includes use of these molecules in the manufacture of a medicament. The nucleic acid may be recombinant, synthetic or produced by any available methods in the art, and the methods include cloning by means of using standard technique.

In some other embodiments, the present invention includes a vector comprising the nucleic acid molecule of the present invention. The vector may be an expression vector, in which the nucleic acid is operatively linked to a control sequence that is able to facilitate the expression of the nucleic acid in a host cell. A plurality of vectors may be used. For example, suitable vectors may include virus (for example poxvirus, adenovirus, baculovirus etc.); or yeast vectors, bacteriophages, chromosomes, artificial chromosomes, plasmids, and cosmids.

In some embodiments, the present invention further includes the cells transfected by these vectors so that the FGFR-Fc fusion protein is expressed. The host cell suitable for the present invention may be a prokaryotic cell or eukaryotic cell. They include bacteria, for example *E. coli*; yeast; insect cells; and mammalian cells. The mammalian cell lines that may be used include, but are not limited to, Chinese Hamster Ovary (CHO) cells, baby hamster kidney cells, NS0 mouse myeloma cells, monkey and human cell lines, and derivative cell lines thereof.

In another aspect of the present invention, a method for angiogenesis inhibition is provided, comprising administering the FGFR-Fc fusion protein of the present invention to the subject in need thereof. Preferably, the method is carried out in a mammal.

In another aspect of the present invention, a method for binding FGF in vitro or in vivo is provided, which comprises contacting FGF to the fusion protein according to the present invention.

In another aspect of the present invention, a method for the treatment or prevention of tumors in a mammal is provided, which comprises administering the FGFR-Fc fusion protein of the present invention to the subject in need thereof. Preferably, the tumor is a solid tumor.

In another aspect of the present invention, a method for the treatment or prevention of ophthalmic angiogenesis-related diseases in a mammal is provided, which comprises administering the FGFR-Fc fusion protein of the present invention to the subject in need thereof. Preferably, the ophthalmic angiogenesis-related disease is age-related macular degeneration.

The present invention also relates to use of the FGFR-Fc fusion protein in the preparation of medicaments for angiogenesis inhibition. Additionally, the present invention also relates to use of the FGFR-Fc fusion protein in the preparation of medicaments for the treatment or prevention of angiogenesis-related diseases. Preferably, angiogenesis-related diseases are tumors or ophthalmic angiogenesis-related disease.

Angiogenesis-related diseases include, but are not limited to, angiogenesis-dependent cancers, including, for example, solid tumors, hematogenic tumors (for example leukemia) and tumor metastasis; benign tumors, for example, angioma, acoustic neuroma, neurofibroma, trachoma and pyogenic granuloma; rheumatoid arthritis; psoriasis; rubeosis; Osler-Webber Syndrome; myocardial angiogenesis; plaque neovascularization; telangiectasia; hemophiliac joint and angiofibroma.

In some embodiments of the methods described, one or more FGFR-Fc fusion proteins may be administered together (simultaneously) or at a different time (sequentially).

Additionally, the fusion protein may be administered together with one or more additional medicaments used for cancer treatment or angiogenesis inhibition.

In some embodiments, the method disclosed in the present invention may be used alone. Alternatively, the subject method may be combined with other conventional anticancer therapies for the treatment or prevention of proliferative diseases (for example tumors). For example, these methods may be used for the prevention of cancers, the prevention of cancer relapse and postoperative metastasis, and may be used as a supplement for other cancer therapies. The effectiveness of conventional cancer therapies (for example, chemotherapy, radiotherapy, phototherapy, immunotherapy and operation) may be enhanced by using target polypeptide therapeutic agents.

In ophthalmology, angiogenesis is related to, for example, diabetic retinopathy, retinopathy of prematurity, age-related macular degeneration, corneal transplantation rejection, neovascular glaucoma and RLF (retrolental fibroplasia). The FGFR-Fc fusion protein disclosed herein can be administered inside the eye or by other routes. Other diseases related to angiogenesis in ophthalmology include, but are not limited to, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sjogren, acne rosacea, phlyctenosis, syphilis, Mycobacteria infection, lipid degeneration, chemical burn, bacterial ulcer, fungal ulcer, Herpes simplex infection, Herpes zoster infection, protozoan infection, Kaposi sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegeners sarcoidosis, Scleritis, Steven's Johnson disease, periphigoid radial keratotomy and corneal graph rejection, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, Eales disease, Bechets disease, infection resulting in retinitis or choroiditis, presumed ocular histoplasmosis, Bests disease, myopia, optic pit, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complication. Other diseases include, but not limited to, rubeosis (neovasculariation of the angle) related diseases and diseases induced by abnormal hyperplasia of the fibrous blood vessel or fibrous tissue, including all kinds of proliferative vitreoretinopathy.

Administration

The fusion protein of the present invention may be administered alone, but preferably, as a pharmaceutical composition, which usually comprises a suitable pharmaceutical excipient, diluent or carrier selected according to the intended administration route. The fusion protein may be administered to the patient in need thereof by any suitable route. A precise dosage will be dependent on many factors, including exact properties of the fusion protein.

Some suitable administration routes include (but are not limited to) oral, rectal, nasal, topical (including buccal and sublingual), subcutaneous, vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intracutaneous, intrathecal and extradural) administration.

For intravenous injection and injection at the focal site, active ingredients are present in the form of a parenterally-acceptable aqueous solution, which is free of pyrogen and has appropriate pH value, isotonicity and stability.

A suitable solution may be well formulated by the skilled one in the art using, for example, isotonic excipients such as sodium chloride injection, Ringer's injection, Ringer's lac-

tate injection. As required, preservative, stabilizer, buffering agent, antioxidant and/or some other additives may be added. The pharmaceutical composition orally administered may be in a form of tablet, capsule, powder or oral liquid etc. Solid carrier, such as gelatin or adjuvant, may be comprised in a tablet. Liquid pharmaceutical composition usually comprises liquid carrier, such as water, petroleum, animal or vegetable oil, mineral oil or synthetic oil. Also included may be normal saline solution, glucose or other sugar solutions or glycols such as ethylene glycol, propylene glycol or polyethylene glycol.

Examples of the techniques and schemes as mentioned above and other techniques and schemes as used according to the present invention may be found in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A. (ed), 1980.

Cloning of the Fusion Protein and Construction of the Expression Plasmid

The FGF receptor fragment can be obtained from the amplification of a cDNA template of a corresponding receptor through PCR. The IgG1 Fc fragment can be obtained from the cDNA amplification of the human-derived IgG1 through PCR. When PCR primers are designed, linking sequences are introduced between different fragments so that these different fragments may be finally linked by overlap PCR to form reading frames for different fusion proteins, and endonuclease BspE I and Pst I sites can be added to both ends of the cDNA. The cDNAs for different fusion proteins may be cloned to the expression plasmid after digestion by BspE I and Pst I. The plasmid after cloning may be determined by endonuclease digestion, electrophoresis and finally DNA sequencing.

Expression and Purification of the Fusion Protein

The present fusion protein may be expressed and purified by techniques commonly used in the art. DNA from corresponding fusion protein plasmid was purified using plasmid purification kit (MAX) available from Qiagen, and the concentration of plasmid DNA can be determined using UV spectrophotometry, and the plasmid was transfected to CHO cell using FUGENE 6 liposome (Roche). Specific methods for transfection can be performed according to the specification of the product.

Based on the expression amount required for the proteins, two methods were employed in the present invention for protein expression: (1) transient expression, in which the fusion protein contained culture supernatant was usually harvested 48-72 h after transfection, and the relative content of the fusion protein was then determined using human IgG ELISA so that the fusion protein may be rapidly and efficiently obtained; (2) establishing a stable cell line and producing the common DHFR-defective CHO cell expression system using the recombinant protein medicament expression, the basic process of which includes cell transfection, selection of stably transfected cell, clone screening, stress amplification, culture medium and process optimization etc., and finally realizing a large-scale suspension culture of CHO engineering cell strain in a serum free culture medium. The culture product was collected and the fusion protein was purified using Protein A affinity column. The purified protein was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and subsequently all eluates in which the required expression product was contained were combined and filtered using a 0.22 μ m filter, and then protein quantification was carried out according to a plurality of methods such as Lowry protein assay. The volume of CHO cell culture in the present invention was at a level of 10 L bioreactor, through which the fusion protein obtained after

purification could satisfy the protein amount required in the animal experiments, and also a basis was established for future scaling-up.

Neutralization of FGF by the Fusion Protein was Validated at a Level of Protein

After the fusion protein expressed by CHO was obtained, the binding capacity of the fusion protein to FGF is evaluated in the present invention at a level of protein. Binding experiment and affinity experiment were performed for validation in the present invention, in which steps of the binding experiment included: after initially coated by FGF-2 on a 96-well ELISA plate, the coated well was blocked by BSA followed by adding each fusion protein at the same concentration, and then a secondary antibody to human IgG Fc-HRP was added after washing, and the samples were developed, stopped and read at 450 nm on a ELISA plate, and finally the fusion protein which had binding capacity to FGF-2 was screened based on the signal strength. The affinity experiment was performed in order to determine the affinity of the fusion protein to FGF-2 in the solution system, which comprised the following steps: FGF-2 was initially coated on a 96-well ELISA plate to capture the antibody, and then the coated well was blocked by BSA, and subsequently a mixture of the fusion protein and FGF-2 which was previously prepared and incubated were added with a gradient of diluted standards, and after incubation, an HRP-labeled detection antibody was added (using antibody 2 which specifically detected free VEGF or FGF-2), and subsequently the samples were developed, stopped and read at 450 nm on a ELISA plate, and finally the relative concentration of free FGF-2 was detected in the mixture of the fusion protein and FGF-2. Through the experiments above, the fusion protein having a blocking effect on FGF-2 was screened.

Neutralization of FGF by the Fusion Protein was Validated at a Cellular Level

After the binding capacity of the fusion protein to FGF-2 was determined at a level of protein, its angiogenesis-inhibiting effect will be further validated at a cellular level in the present invention. The inhibition capacity of the fusion protein on the division and migration of the vascular endothelial cell is examined by the division test using human umbilical vein endothelial cell (HUVEC) and the HUVEC cell migration test. The inhibition capacity of the fusion protein on the division of HUVEC cell can be examined by the HUVEC cell division test, which comprises the following steps during the experiment: 3000 HUVEC cells/well were inoculated to a 96-well plate and cultured at 37° C. in an incubator supplemented with 5% CO₂, and then FGF-2 as well as a mixture of the fusion protein at different concentrations with FGF-2 are added respectively, and after culturing for another 3-4 days, 10% CCK-8 is added and cultured for 2 h before the sample is read at 450 nm on a ELISA plate. The inhibition capacity of the fusion protein on the division of vascular endothelial cell induced by FGF-2 was evaluated based on the difference of absorbance, and the median effective concentration of the fusion protein was obtained for FGF-2 inhibition. The inhibition capacity of the fusion protein on HUVEC cell migration was examined by the HUVEC cell migration test, which comprises the following steps during the experiment: 50000 HUVEC cells as well as the fusion protein at various concentrations were initially inoculated in the upper chamber, while 600 μ L FGF-2 containing culture liquid was added into the lower chamber, and subsequently, the sample was cultured at 37° C. in an incubator supplemented with 5% CO₂ for 20-24 h before cells on the face side of the membrane of the upper chamber were removed, and then cells on the back side of the membrane were fixed, stained and washed with PBS before

observed and counted under an inverted microscope. The migration of HUVEC cells induced by the stimulation of FGF-2 was demonstrated by counting the HUVEC cells on the back side of the membrane, and the inhibition capacity of the fusion protein on the migration of the vascular endothelial cell was tested by adding the fusion protein at various concentrations into the culture liquid. Through the experiments mentioned above, the inhibition capacity of the new fusion protein constructed in the present invention was validated on the division and migration of the vascular endothelial cell induced by FGF-2, which also provided a basis for future animal experiments.

Tumor Growth-Inhibiting Capacity of the Fusion Protein was Validated by the Tumor Model

After the blocking effect of the new fusion protein in the present invention on FGF-2 signal was demonstrated by experiments at a protein level and a cellular level, its anti-tumor capacity would be tested in animal tumor models in the present invention. In the present invention, the anti-angiogenesis and anti-tumor effect of the fusion protein would be validated by models commonly used in searching medicaments for angiogenesis and tumor, for example, LLC mouse lung cancer, U87 gliocytoma, B 16 melanoma and so on. In animal experiments, in addition to conventional control groups, control medicaments, such as VEGF-Trap, FP-1039, would also be included so as to obtain comparative data for anti-tumor capacity. During experiments, 100 μ L tumor cell liquid with appropriate amount was subcutaneously injected into C57 mouse on one side of the back, and the tumor volume was measured with a vernier caliper twice a week. Upon the tumor grew to about 200 mm³, the fusion protein at various concentrations was subcutaneously injected and the mice were sacrificed after 2-3 weeks. Subsequently, the tumor volume was measured with a vernier caliper, and the anti-tumor effect of the fusion protein was validated by the size of the tumor. Furthermore, individual tumor tissue was analyzed using methods such as immunohistochemistry to investigate the regulation mechanism of angiogenesis.

EXAMPLES

Example 1

Construction of Recombinant Expression Plasmid for FGFR1-Fc Fusion Protein

The FGF receptor fragment is obtained from the amplification of the cDNA templet of FGF receptor through PCR, and IgG1 Fc fragment is obtained from the cDNA amplification of the human-derived IgG1 through PCR. A commercially available cDNA (PCR Ready First Strand cDNA, derived from human adult colon cancer tissue, BioChain) was used as the template for FGFR1 fragment. Total RNA was extracted from the blood of healthy human subjects using human blood RNA extraction kit (QIAGEN). According to the manufacturer's instruction of reverse transcription kit (Promega), RT-PCR was performed using M-MLV reverse transcriptase (Promega) so that RNA was reversely transcribed to cDNA which was used as the template for IgG1 Fc fragment. RT-PCR was performed according to the manufacturer's instruction of reverse transcription kit, which has the following steps: Oligo dT, dNTP, total RNA and DEPC H₂O were mixed homogeneously and reacted at 70° C. for 10 min before placed on ice for 5 min, and subsequently RNase inhibitor, M-MLV reverse transcriptase and reaction buffer

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were added. The mixture was reacted at 42° C. for 1 h and subsequently at 70° C. for 15 min, and the cDNA obtained may be used as the template.

Various FGFR1 fragments were individually amplified by PCR using the cDNA from human adult colon cancer tissue as the template (the primers were listed in table 1), and IgG1 Fc fragment was amplified by PCR using human blood cDNA as the template (the primers were listed in table 1 and 2). The reaction conditions for the PCR were as follows: 5 min of pre-denaturalization at 98° C., total 30 cycles of 30 s of denaturalization at 98° C., 45 s of annealing at 56° C. and 2 min of extension at 72° C., and finally another 10 min of extension. When PCR primers were designed, 20 or more complementary base sequences were introduced as the linking sequence between FGFR1 fragment and IgG1 Fc fragment so that the FGFR1 fragment and IgG1 Fc fragment may be subsequently linked by overlap PCR to form reading frames for different fusion proteins, and at the same time, restriction endonuclease BspE I and Pst I site were added at both ends of the PCR product.

Subsequently, overlap PCR was carried out to obtain each FGFR1-Fc fusion protein fragment by amplification. The process of the overlap PCR reaction may be divided into two rounds, in which the fragment required for linking and containing no primer was included in the first round with reaction conditions as follows: 5 min of pre-denaturalization at 98° C.,

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Pst I, respectively. Subsequently, 1% agarose gel electrophoresis was performed on the digested samples under a voltage of 90 V. Target fragments were recovered using QIAquick gel extraction kit (QIAGEN) before ligating at 16° C. for 1 h using a ligase (NEB). The mixture for ligation reaction was transformed to the competent Top10 *E. coli* under the conditions of 90 s of reaction at 42° C. followed by 3 min of standing on ice. After the sterile LB culture broth (free of antibody) added, the mixture was shaken at 250 rpm in a shaker at 37° C. for 1 h before coating on a LB plate supplemented with ampicillin. The plate was cultured overnight in a thermostated incubator at 37° C., and then single colonies were picked out and transferred to an ampicillin-containing LB culture broth. The inoculated culture broth was shaken at 250 rpm in a shaker at 37° C. overnight before the plasmid was extracted using alkaline lysis. Subsequently, the sample was digested by restriction endonuclease before evaluated by 1% agarose gel electrophoresis under a voltage of 90 V. The recombinant plasmid with correct endonuclease digestion was confirmed by DNA sequencing. Based on the steps above, 19#, 13#, 22#, 23#, 26#, 29# and 8# expression plasmid for FGFR1-Fc fusion protein were constructed. The protein sequence of FGFR1-Fc in each fusion protein and its encoding nucleotide sequence were listed in Table 3. The schematic diagram of the fusion protein structure was shown in FIG. 1.

TABLE 1

Primers used for amplification of FGFR1 fragment		
Fusion protein	Upstream primer	Downstream primer
19#	19#-FGFR1For (SEQ ID NO: 24) TAGTTCCGGAAGGCCGTCCTCCGACCTTGCCCTG	FGFR1Rev (SEQ ID NO: 31) GTTTGTCTCTCCAGGTAC AGGGGCGAGGTC
13#	13#-FGFR1For (SEQ ID NO: 25) TAGTTCCGGAAGGAAATCGCACCCGCATCACAG	FGFR1Rev
22#	22#-FGFR1For (SEQ ID NO: 26) TAGTTCCGAGTAACAGCAGCCCTCGGGC	FGFR1Rev
23#	23#-FGFR1For (SEQ ID NO: 27) TAGTTCCGATCCTCTTCAGAGGAGAAAGAAAC	FGFR1Rev
26#	26#-FGFR1For (SEQ ID NO: 28) TAGTTCCGGAAGAACCTAACCCGTAGCTCCAT	FGFR1Rev
29#	29#-FGFR1For (SEQ ID NO: 29) TAGTTCCGACCATATTGGACATCCCAGAAAG	FGFR1Rev
8#	8#-FGFR1For (SEQ ID NO: 30) CTAGTCCGGACAGAAAGATGGAAAGAAATTGC	FGFR1Rev

6 cycles of 30 s of denaturalization at 98° C., 45 s of annealing at 56° C. and 5 min of extension at 72° C., and finally another 10 min of extension at 72° C.; after the first round, the second round of PCR was carried out by adding the primers for both ends with reaction conditions as follows: 5 min of pre-denaturalization at 98° C., 30 cycles of 30 s of denaturalization at 98° C., 45 s of annealing at 56° C. and 2 min of extension at 72° C., and finally another 10 min of extension at 72° C.; through the process above, reading frames for different fusion proteins were spliced, and at the same time, restriction endonuclease BspE I and Pst I site were added at both ends of the cDNA.

After amplification, the fragments amplified by PCR were purified using QIAquick PCR purification kit (QIAGEN). cDNAs of various fusion proteins and the eucaryotic expression plasmid pSV2-dhfr (ATCC) were digested by BspE I and

TABLE 2

Primers used for amplification of IgG1 Fc fragment		
	Upstream primer	Downstream primer
IgG1 Fc fragment	FcFor (SEQ ID NO: 32) CTGTACCTGGAGGACAAACT CACACATGC	FcRev (SEQ ID NO: 33) GATATCTGCAGTCATTT ACCCGGAGACAGG

TABLE 3

Protein sequences and nucleotide sequences for FGFR1-Fc fusion proteins		
Fusion protein	Protein Sequence	Nucleotide Sequence
19#	SEQ ID NO: 9	SEQ ID NO: 16
13#	SEQ ID NO: 10	SEQ ID NO: 17
22#	SEQ ID NO: 11	SEQ ID NO: 18
23#	SEQ ID NO: 12	SEQ ID NO: 19
26#	SEQ ID NO: 13	SEQ ID NO: 20
29#	SEQ ID NO: 14	SEQ ID NO: 21
8#	SEQ ID NO: 15	SEQ ID NO: 22

Example 2

Transient Expression and Quantification of the Fusion Proteins

The DNA of individual fusion protein plasmid was purified using MAX Plasmid Purification Kit (Qiagen). The concentration of the plasmid DNA was determined by UV spectrophotometry. 1 µg recombinant plasmid and 6 µL liposome (FuGENE 6 Transfection Reagent, Roche) were homogeneously mixed into 100 µL fresh IMDM culture broth (GIBCO); after standing for 15 min, the mixture was added to the CHO cells (ATCC) cultured overnight after inoculation at a cell density of 3×10^5 /mL into a 6-well plate; the mixture was cultured at 37° C. in an incubator supplemented with 5% CO₂ for 48 h with a cell complete culture broth (IMDM medium containing 10% FBS, 1% HT and 1% glutamine, all supplied by GIBCO); subsequently, the supernatant was collected and determined for the relative content of the fusion protein using human IgG ELISA kit for protein quantification (BETHYL). The relative content of the fusion protein expressed and secreted by CHO was determined with the following steps: 100 µL anti-human IgG-Fc protein (10 µg/mL) purified by affinity was coated to a 96-well ELISA plate (IMMULON) and subsequently washed for 5 times using 300 µL PBST washing solution; each coated well was blocked with 200 µL freshly prepared blocking working solution (blocking stock solution: PBS=1:19) and incubated at 37° C. for 1 h; after washed in 300 µL PBST washing solution for 5 times, 100 µL IgG solution diluted in a gradient (200 ng/mL original concentration and diluted by PBS in the multiple proportion of 1:2) as a standard and 100 µL culture supernatant of each fusion protein diluted in a gradient (starting with the concentration of each culture supernatant, and diluted by PBS in the multiple proportion of 1:5) were added to each well and incubated at 37° C. for 2 h; after washed in 300 µL PBST washing solution for 5 times, 100 µL anti-human IgG Fc-HRP secondary antibodies diluted with PBS in a ratio of 1:10000 was added and incubated at 37° C. for 1 h; after washed, the well was developed by adding 100 µL developing solution (KPL); finally, after the development was stopped by adding 100 µL stopping solution (KPL), the absorbance of the ELISA plate was read at a wavelength of 450 nm on a ELISA reader. The concentrations of various fusion proteins may thereby be determined according to the standard curve.

Example 3

Binding of the Fusion Proteins

The binding capacity of 19#, 13#, 22#, 23#, 26#, 29# and 8# fusion protein constructed above to FGF-2 was detected by ELISA.

Initially, a 96-well ELISA plate (IMMULON Company) was coated by 100 µL solution containing 50 ng/mL FGF-2 (R&D Systems) as well as containing 100 ng/mL heparin (Sigma Company) and 50 ng/mL FGF-2. Subsequently, the plate was washed by 300 µL PBST washing solution for 5 times before each coated well was blocked by 200 µL freshly prepared blocking working solution (KPL Company) (blocking stock solution: PBS=1:19) and incubated at 37° C. for 1 h. After washed in 300 µL PBST washing solution for 5 times, 100 µL solutions of various fusion proteins (dissolve in PBS, pH=7.2, concentration of 20 ng/mL) were added and incubated at 37° C. for 2 h. After washed in 300 µL PBST washing solution for 5 times, 100 µL secondary antibody to human IgG Fc-HRP (BETHYL Company) diluted with PBS in a ratio of 1:10000 was added and incubated at 37° C. for 1 h. After washing in 300 µL PBST washing solution 5 times, the well was developed to the presence of color at room temperature in a dark place by adding 100 µL developing solution (KPL Company), and finally the development was stopped by adding 100 µL stopping solution (KPL Company) before the absorbance of the ELISA plate was read at a wavelength of 450 nm on a ELISA reader.

The higher the binding capacity of the fusion protein to FGF2, the larger the absorbance and the stronger the signal. Based on the strength of the signal, 26# fusion protein was determined to have the highest binding capacity to FGF-2.

Comparison of FGF-2 binding among various fusion proteins is shown in FIG. 2. It can be seen from FIG. 2 that 19#, 13#, 22#, 23#, 26# and 29# fusion protein bound to FGF at different extents in the presence of heparin, and particularly, the binding extent of 23#, 26# and 29# was extremely higher than control, and higher than that of 19#, 13# and 22#, indicating that the fusion proteins containing no acidic box had excellent effect.

Among others, especially high binding extent was demonstrated by 26#, indicating that the fusion protein had significantly better binding effect when it comprises a part of certain length derived from the intermediate functional sequence of the Ig-like domain of FGFR.

Example 4

Stable Expression and Purification of the Fusion Proteins

DHFR-defective CHO cells (ATCC) were transfected by the recombinant expression plasmid of 26# fusion protein (possessing a high FGF-2 binding capacity) through a liposome (Roche).

Particularly, 5 µg recombinant plasmid and 30 µL liposome (FuGENE 6 Transfection Reagent, Roche) were homogeneously mixed into 100 µL fresh IMDM culture broth (GIBCO); after standing for 15 min, the mixture was added to the DHFR-defective CHO cells (ATCC) cultured overnight after inoculation at a cell density of 3×10^5 /mL in a 10 cm culture dish (Corning); the mixture was cultured at 37° C. in an incubator supplemented with 5% CO₂ for 2-3 days with a cell complete culture broth containing 10% FBS, 1% HT and 1% glutamine in a IMDM culture medium (all supplied by GIBCO); subsequently, the cells were digested by trypsin (GIBCO), inoculated at a cell density of 3×10^5 /mL in 30 mL serum-free 302 culture medium (SAFC) in a flask, and selectively cultured at 37° C. in an incubator supplemented with 5% CO₂ at 100 rpm to a cell density of 10^6 /mL.

Subsequently, 3000 cells were inoculated into a 10 cm culture dish (Corning) (the culture broth containing 10% FBS

and 1% glutamine in an IMDM culture medium) and cultured at 37° C. in an incubator supplemented with 5% CO₂ to form single clones.

These single clones were picked out and cultured in a 96-well plate (Corning). The relative content of the fusion protein expressed and secreted by each individual single clone was determined using a human IgG ELISA kit for protein quantification (BETHYL) under the same conditions and steps as described in Example 2 for the determination of the relative content of the fusion protein. The clone with the highest expression amount was screened out and transferred to a 6-well plate for culturing to a confluence rate of about 70%. The cells were digested by trypsin and transferred to a 10 cm culture dish. Subsequently, gradual stress amplification was carried out by adding methotrexate (MTX, Sigma) with various concentrations (10 nM, 20 nM, 50 nM, 100 nM, 200 nM and 500 nM). After stress amplification, the cells were digested by trypsin and inoculated at a cell density of 3×10⁵/mL in a flask. The expression amount of a single cell was determined so that genetically engineered strains of CHO were obtained for expressing a particular fusion protein. Finally, large-scale suspension culture (volume of 10 L) of the genetically engineered strain of CHO was carried out at 37° C., 5% CO₂, 40% dissolved oxygen and 80 rpm in a serum-free 302 culture medium (pH 7.0, SAFC). The culture product was collected by centrifugation. After the supernatant was filtered using 0.45 μm filter membrane (Millipore), affinity chromatography was performed according to the instruction manual of Protein A affinity column (GE) with the specific steps as follows: initially, a protein A affinity column was equilibrated by a PBS buffer (pH 7.0); subsequently, the supernatant was loaded on the column and washed again with the PBS buffer; finally, the column was eluted with a citric acid buffer (pH 3.0), and the eluent was collected and filtered by a 0.45 μm filter membrane. After virus inactivation by adding S/D (0.3% tributyl phosphate/1% Tween 80) at 24° C. for 6 h, the target protein was further purified by a molecular sieve chromatography with the following steps: first, the eluent obtained from the Protein A affinity chromatography was dialyzed in a dialysis bag against a PBS buffer; subsequently, the sample was concentrated in a 10 KD ultrafiltration cup (Millipore); the sample concentrated using the ultrafiltration cup was then loaded on a molecular sieve chromatography column Superdex 200 (GE) equilibrated by a PBS buffer, and subsequently the column was eluted with a PBS buffer and the eluting peak was collected. The purified protein was analyzed by SDS-PAGE (FIG. 3); and subsequently, the eluates containing the required expression product was combined and filtered with a 0.22 μm filter membrane (Millipore) before the protein content was determined using many methods such as Lowry protein assay.

Example 5

Gradient-Binding Experiment of the Fusion Proteins

The binding capacities of the fusion proteins as constructed above to FGF-2 were detected by ELISA, similarly as in Example 3.

Initially, a 96-well ELISA plate was coated by 100 μL solution containing 50 ng/mL FGF-2 (R&D Systems). Subsequently, the plate was washed in 300 μL PBST washing solution for 5 times before each coated well was blocked by 200 μL freshly prepared blocking working solution (KPL) (blocking stock solution:PBS=1:19) and incubated at 37° C. for 1 h. After washed in 300 μL PBST washing solution for 5 times, 100 μL solutions containing various fusion proteins at

different concentrations (the starting content of protein was 16000 pM, and was diluted in a ratio of 1:3) were added and incubated at 37° C. for 2 h. After washed in 300 PBST washing solution for 5 times, 100 μL anti-human IgG Fc-HRP secondary antibody (BETHYL) diluted with PBS in a ratio of 1:10000 was added and incubated at 37° C. for 1 h. After washed in 300 μL PBST washing solution for 5 times, the well was developed by adding 100 μL developing solution (KPL), and finally the development was stopped by adding 100 μL stopping solution (KPL) before the absorbance of the ELISA plate was read at a wavelength of 450 nm on a ELISA reader. Based on the intensity of the signal, the gradient binding capacities of the fusion proteins to FGF-2 were determined. In the experiment procedure mentioned above, specific conditions and steps may be found in Example 3. Gradient binding of 26# fusion protein to FGF-2 was compared in FIG. 4. It can be seen that the binding capacity of 26# fusion protein to FGF-2 was dose-dependent.

It has been suggested by this example that the binding capacity to FGF-2 increased with an enhanced molar concentration of 26# fusion protein, manifested by a stronger signal at a wavelength of 450 nm; while the binding capacity to FGF-2 decreased correspondingly with a gradient dilution of the molar concentration of 26# fusion protein.

Example 6

Affinity Experiment of the Fusion Proteins

The affinity of the fusion protein to FGF-2 in a solution system was determined by an affinity experiment.

Initially, a 96-well ELISA plate was coated by 100 μL solution containing 2.0 μg/mL FGF-2 capture antibody (R&D Systems). Subsequently, the plate was washed in 300 μL PBST washing solution for 5 times before each coated well was blocked by a blocking working solution (KPL) (as seen in Example 3) and incubated at 37° C. for 1 h. After washed in 300 μL PBST washing solution for 5 times, previously prepared and incubated (4° C. overnight) mixture of the fusion proteins and FGF-2 as well as the standard (R&D Systems) diluted in a gradient were added, in which the specific preparation procedure was as follows: the starting concentration of 26# fusion protein was 400 pM (dissolved in PBS) and diluted in a gradient ratio of 2-fold, and the solutions of the fusion protein were 1:1 mixed with 20 pM FGF-2 solution (dissolved in PBS), and that is, the starting final concentration of each fusion protein was 200 pM, and the final concentration of FGF-2 was 10 pM in the mixture solution prepared. The plate was incubated at 37° C. for 2 h and washed in 300 μL PBST washing solution for 5 times before 100 μL FGF-2 detection antibody solution (250 ng/mL) was added (R&D systems, which may specifically detect free antibodies against FGF-2). The plate was incubated at 37° C. for 2 h and washed in 300 μL PBST washing solution for 5 times, and subsequently, HRP labeled streptavidin (R&D systems) was added (diluted by PBS in 1:200). The plate was incubated at 37° C. for 2 h and washed in 300 μL PBST washing solution for 5 times before the well was developed at room temperature in a dark place for an appropriate duration (about 15-30 min) by adding 100 μL developing solution (KPL). Finally, after the development was stopped by adding 100 μL stopping solution (KPL), the absorbance of the ELISA plate was read at a wavelength of 450 nm on a ELISA reader. The relative concentration of free FGF-2 in the mixture of the fusion protein and FGF-2 was determined. The affinity between 26# fusion protein and FGF-2 in a solution system can be seen in FIG. 5. As demonstrated in this

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Example, 26# fusion protein had high affinity to FGF-2 in a solution system. The affinity increased with an enhanced concentration, which is manifested as a decreased amount of free FGF-2 with an enhanced concentration of the fusion protein. The affinity between 26# fusion protein and FGF-2 in a solution system can be seen in FIG. 5. As demonstrated in this Example, 26# fusion protein had affinity to FGF-2 in a solution system. The affinity increased with an enhanced concentration, which is manifested as a decreased amount of free FGF-2.

Example 7

Inhibitory Test for Division on Human Umbilical Vein Endothelial Cell

The inhibitory ability of the fusion proteins on the division of vascular endothelial cells was examined in a division test for human umbilical vein endothelial cell (HUVEC).

HUVEC cells (AllCells) were cultured to the exponential growth phase in an HUVEC complete medium (AllCells) at 37° C. in an incubator supplemented with 5% CO₂. HUVEC cells were counted after digested by trypsin. 3000 HUVEC cells were inoculated per well in an HUVEC basal medium containing 1% FBS (AllCells) in a 96-well plate. The plate was cultured overnight at 37° C. in an incubator supplemented with 5% CO₂.

100 μ L FGF-2 (R&D Systems) solution (final concentration of 5 ng/mL) diluted by an HUVEC basal medium containing 1% FBS, as well as 100 μ L mixture of various amount of 26# fusion protein and FGF-2 (in which the final concentration of the fusion protein was 40 pM, diluted in an HUVEC basal medium containing 1% FBS with a ratio of 1:10, and the final concentration of FGF-2 was 5 ng/mL) were added and cultured for another 3-4 days. Subsequently, the culture medium was taken out and a culture medium containing 10% CCK-8 (DOJINDO) was added for another 2 h of culture before the absorbance of the 96-well plate was read directly at a wavelength of 450 nm on an ELISA reader. Based on the difference of the absorbance, the inhibitory ability of the fusion protein on the division of vascular endothelial cells induced by FGF-2 was determined. The effect of the fusion protein on HUVEC cell division induced by FGF-2 was shown in FIG. 6. As demonstrated in this Example, 26# fusion protein has biological activity and function at the cellular level, which can inhibit HUVEC cell division induced by FGF-2, and has the binding capacity to FGF-2. Such binding capacity increases as the molar concentration of 26# fusion protein increases, which is indicated by the inhibition of HUVEC cell division induced by FGF-2.

Example 8

Anti-Tumor Efficacy of FGFR-Fc in Renal Carcinoma Model

Human renal carcinoma cell line Caki-1 cells (2×10^6 cells/mouse) and human lung carcinoma cell line A549 cells (5×10^6 cells/mouse) were suspended in serum-free medium and s.c. injected into the right flanks of 6 to 8 weeks old female, athymic BALB/c nu/nu mice. Tumor volume was calculated twice a week with a caliper by the formula of $[(\text{length} \times \text{width} \times \text{width})/2]$. When tumor size reached around 50~100 mm³, animals were randomized into four groups and received a s.c. injection of FGFR-Fc (#26 fusion protein) at a dose of 25, 2.5, 0.25 mg/kg and PBS twice weekly for 6 to 8

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weeks. 3 days after the last dose, animals were sacrificed and tumors were measured. The results are shown in FIG. 7.

Example 9

Anti-Tumor Efficacy of FGFR-Fc in Lung Carcinoma Model

Effect of FGFR-Fc (#26 fusion protein) on Caki-1 and A549 tumor growth in vivo. Caki-1 cells (2×10^6 ; A) and A549 cells (5×10^6 ; B) were s.c. injected into BALB/c nu/nu mice. FGFR-Fc blocked the growth of indicated s.c. implanted tumors, at the indicated doses twice weekly for 6 to 8 weeks. The tumor volumes $[(\text{length} \times \text{width} \times \text{width})/2]$ were measured, error bars represent standard error of mean, n=6-8 mice/treatment group. The results are shown in FIG. 8.

The present invention has been illustrated by specific examples. However, it will be appreciated by a person of ordinary skill in the art that the present invention is not limited to the specific embodiments. Various changes and modifications may be made by a person of ordinary skill under the scope of the present invention, and each technical feature mentioned in the specification may be combined without departing from the spirit and scope of the invention. Such changes and modifications fall within the scope of the present invention.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

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SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 33

<210> SEQ ID NO 1
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 <223> OTHER INFORMATION: amino acid sequence of human FGFR1
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 <223> OTHER INFORMATION: Ig3_FGFR

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 20             25             30

Pro Trp Gly Ala Pro Val Glu Val Glu Ser Phe Leu Val His Pro Gly
 35             40             45

Asp Leu Leu Gln Leu Arg Cys Arg Leu Arg Asp Asp Val Gln Ser Ile
 50             55             60

Asn Trp Leu Arg Asp Gly Val Gln Leu Ala Glu Ser Asn Arg Thr Arg
 65             70             75             80

Ile Thr Gly Glu Glu Val Glu Val Gln Asp Ser Val Pro Ala Asp Ser
 85             90             95

Gly Leu Tyr Ala Cys Val Thr Ser Ser Pro Ser Gly Ser Asp Thr Thr
100             105             110

Tyr Phe Ser Val Asn Val Ser Asp Ala Leu Pro Ser Ser Glu Asp Asp
115             120             125

Asp Asp Asp Asp Asp Ser Ser Ser Glu Glu Lys Glu Thr Asp Asn Thr
130             135             140

Lys Pro Asn Pro Val Ala Pro Tyr Trp Thr Ser Pro Glu Lys Met Glu
145             150             155             160

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Lys 165	Lys	Leu	His	Ala	Val	Pro	Ala	Lys	Thr	Val	Lys	Phe	Lys	Cys
Pro 180	Ser	Ser	Gly	Thr	Pro	Asn	Pro	Thr	Leu	Arg	Trp	Leu	Lys	Gly
Lys 195	Glu	Phe	Lys	Pro	Asp	His	Arg	Ile	Gly	Gly	Tyr	Lys	Val	Tyr
Ala 210	Thr	Trp	Ser	Ile	Ile	Met	Asp	Ser	Val	Val	Pro	Ser	Asp	Gly
Asn 225	Tyr	Thr	Cys	Ile	Val	Glu	Asn	Glu	Tyr	Gly	Ser	Ile	Asn	Thr
Tyr	Gln	Leu	Asp	Val	Val	Glu	Arg	Ser	Pro	His	Arg	Pro	Ile	Gln
Ala	Gly	Leu	Pro	Ala	Asn	Lys	Thr	Val	Ala	Leu	Gly	Ser	Asn	Glu
Phe 275	Met	Cys	Lys	Val	Tyr	Ser	Asp	Pro	Gln	Pro	His	Ile	Gln	Leu
Lys 290	His	Ile	Glu	Val	Asn	Gly	Ser	Lys	Ile	Gly	Pro	Asp	Asn	Pro
Tyr 305	Val	Gln	Ile	Leu	Lys	Thr	Ala	Gly	Val	Asn	Thr	Thr	Asp	Glu
Met	Glu	Val	Leu	His	Leu	Arg	Asn	Val	Ser	Phe	Glu	Asp	Ala	Glu
Tyr	Thr	Cys	Leu	Ala	Gly	Asn	Ser	Ile	Gly	Leu	Ser	His	His	Ala
Trp	Leu	Thr	Val	Leu	Glu	Ala	Leu	Glu	Glu	Arg	Pro	Ala	Val	Thr
Ser 370	Pro	Leu	Tyr	Leu	Glu	Ile	Ile	Ile	Tyr	Cys	Thr	Gly	Ala	Leu
Ile 385	Ser	Cys	Met	Val	Gly	Ser	Val	Ile	Val	Tyr	Lys	Met	Lys	Gly
Thr	Lys	Lys	Ser	Asp	Phe	His	Ser	Gln	Met	Ala	Val	His	Lys	Ala
Lys	Ser	Ile	Pro	Leu	Arg	Arg	Gln	Val	Thr	Val	Ser	Ala	Asp	Ser
Ala	Ser	Met	Asn	Ser	Gly	Val	Leu	Leu	Val	Arg	Pro	Ser	Arg	Ser
Ser 450	Ser	Gly	Thr	Pro	Met	Leu	Ala	Gly	Val	Ser	Glu	Tyr	Glu	Pro
Glu 465	Asp	Pro	Arg	Trp	Glu	Leu	Pro	Arg	Asp	Arg	Leu	Val	Leu	Lys
Pro	Leu	Gly	Glu	Gly	Cys	Phe	Gly	Gln	Val	Val	Leu	Ala	Glu	Ile
Gly	Leu	Asp	Lys	Asp	Lys	Pro	Asn	Arg	Val	Thr	Lys	Val	Ala	Lys
Met	Leu	Lys	Ser	Asp	Ala	Thr	Glu	Lys	Asp	Leu	Ser	Asp	Leu	Ser
Glu 530	Met	Glu	Met	Met	Lys	Met	Ile	Gly	Lys	His	Lys	Asn	Ile	Asn
Leu 545	Leu	Gly	Ala	Cys	Thr	Gln	Asp	Gly	Pro	Leu	Tyr	Val	Ile	Glu
Tyr	Ala	Ser	Lys	Gly	Asn	Leu	Arg	Glu	Tyr	Leu	Gln	Ala	Arg	Pro

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Pro Gly Leu Glu Tyr Cys Tyr Asn Pro Ser His Asn Pro Glu Glu Gln
 580 585 590
 Leu Ser Ser Lys Asp Leu Val Ser Cys Ala Tyr Gln Val Ala Arg Gly
 595 600 605
 Met Glu Tyr Leu Ala Ser Lys Lys Cys Ile His Arg Asp Leu Ala Ala
 610 615 620
 Arg Asn Val Leu Val Thr Glu Asp Asn Val Met Lys Ile Ala Asp Phe
 625 630 635 640
 Gly Leu Ala Arg Asp Ile His His Ile Asp Tyr Tyr Lys Lys Thr Thr
 645 650 655
 Asn Gly Arg Leu Pro Val Lys Trp Met Ala Pro Glu Ala Leu Phe Asp
 660 665 670
 Arg Ile Tyr Thr His Gln Ser Asp Val Trp Ser Phe Gly Val Leu Leu
 675 680 685
 Trp Glu Ile Phe Thr Leu Gly Gly Ser Pro Tyr Pro Gly Val Pro Val
 690 695 700
 Glu Glu Leu Phe Lys Leu Leu Lys Glu Gly His Arg Met Asp Lys Pro
 705 710 715 720
 Ser Asn Cys Thr Asn Glu Leu Tyr Met Met Met Arg Asp Cys Trp His
 725 730 735
 Ala Val Pro Ser Gln Arg Pro Thr Phe Lys Gln Leu Val Glu Asp Leu
 740 745 750
 Asp Arg Ile Val Ala Leu Thr Ser Asn Gln Glu Tyr Leu Asp Leu Ser
 755 760 765
 Met Pro Leu Asp Gln Tyr Ser Pro Ser Phe Pro Asp Thr Arg Ser Ser
 770 775 780
 Thr Cys Ser Ser Gly Glu Asp Ser Val Phe Ser His Glu Pro Leu Pro
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 Leu Lys Arg Arg
 820

<210> SEQ ID NO 2
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 <223> OTHER INFORMATION: amino acid sequence of human VEGFR1

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 Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr
 35 40 45
 Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
 50 55 60
 Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
 65 70 75 80
 Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
 85 90 95
 Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val

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100							105					110				
Pro	Thr	Ser	Lys	Lys	Lys	Glu	Thr	Glu	Ser	Ala	Ile	Tyr	Ile	Phe	Ile	
		115					120					125				
Ser	Asp	Thr	Gly	Arg	Pro	Phe	Val	Glu	Met	Tyr	Ser	Glu	Ile	Pro	Glu	
		130					135					140				
Ile	Ile	His	Met	Thr	Glu	Gly	Arg	Glu	Leu	Val	Ile	Pro	Cys	Arg	Val	
		145					150					155				
Thr	Ser	Pro	Asn	Ile	Thr	Val	Thr	Leu	Lys	Lys	Phe	Pro	Leu	Asp	Thr	
Leu	Ile	Pro	Asp	Gly	Lys	Arg	Ile	Ile	Trp	Asp	Ser	Arg	Lys	Gly	Phe	
Ile	Ile	Ser	Asn	Ala	Thr	Tyr	Lys	Glu	Ile	Gly	Leu	Leu	Thr	Cys	Glu	
Ala	Thr	Val	Asn	Gly	His	Leu	Tyr	Lys	Thr	Asn	Tyr	Leu	Thr	His	Arg	
Gln	Thr	Asn	Thr	Ile	Ile	Asp	Val	Gln	Ile	Ser	Thr	Pro	Arg	Pro	Val	
Lys	Leu	Leu	Arg	Gly	His	Thr	Leu	Val	Leu	Asn	Cys	Thr	Ala	Thr	Thr	
Pro	Leu	Asn	Thr	Arg	Val	Gln	Met	Thr	Trp	Ser	Tyr	Pro	Asp	Glu	Lys	
Asn	Lys	Arg	Ala	Ser	Val	Arg	Arg	Arg	Ile	Asp	Gln	Ser	Asn	Ser	His	
Ala	Asn	Ile	Phe	Tyr	Ser	Val	Leu	Thr	Ile	Asp	Lys	Met	Gln	Asn	Lys	
Asp	Lys	Gly	Leu	Tyr	Thr	Cys	Arg	Val	Arg	Ser	Gly	Pro	Ser	Phe	Lys	
Ser	Val	Asn	Thr	Ser	Val	His	Ile	Tyr	Asp	Lys	Ala	Phe	Ile	Thr	Val	
Lys	His	Arg	Lys	Gln	Gln	Val	Leu	Glu	Thr	Val	Ala	Gly	Lys	Arg	Ser	
Tyr	Arg	Leu	Ser	Met	Lys	Val	Lys	Ala	Phe	Pro	Ser	Pro	Glu	Val	Val	
Trp	Leu	Lys	Asp	Gly	Leu	Pro	Ala	Thr	Glu	Lys	Ser	Ala	Arg	Tyr	Leu	
Thr	Arg	Gly	Tyr	Ser	Leu	Ile	Ile	Lys	Asp	Val	Thr	Glu	Glu	Asp	Ala	
Gly	Asn	Tyr	Thr	Ile	Leu	Leu	Ser	Ile	Lys	Gln	Ser	Asn	Val	Phe	Lys	
Asn	Leu	Thr	Ala	Thr	Leu	Ile	Val	Asn	Val	Lys	Pro	Gln	Ile	Tyr	Glu	
Lys	Ala	Val	Ser	Ser	Phe	Pro	Asp	Pro	Ala	Leu	Tyr	Pro	Leu	Gly	Ser	
Arg	Gln	Ile	Leu	Thr	Cys	Thr	Ala	Tyr	Gly	Ile	Pro	Gln	Pro	Thr	Ile	
Lys	Trp	Phe	Trp	His	Pro	Cys	Asn	His	Asn	His	Ser	Glu	Ala	Arg	Cys	
Asp	Phe	Cys	Ser	Asn	Asn	Glu	Glu	Ser	Phe	Ile	Leu	Asp	Ala	Asp	Ser	
Asn	Met	Gly	Asn	Arg	Ile	Glu	Ser	Ile	Thr	Gln	Arg	Met	Ala	Ile	Ile	
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Gly	Arg	Asn	Ile	Ser	Phe	Tyr	Ile	Thr	Asp	Val	Pro	Asn	Gly	Phe	His
545					550					555					560
Val	Asn	Leu	Glu	Lys	Met	Pro	Thr	Glu	Gly	Glu	Asp	Leu	Lys	Leu	Ser
				565					570					575	
Cys	Thr	Val	Asn	Lys	Phe	Leu	Tyr	Arg	Asp	Val	Thr	Trp	Ile	Leu	Leu
			580					585					590		
Arg	Thr	Val	Asn	Asn	Arg	Thr	Met	His	Tyr	Ser	Ile	Ser	Lys	Gln	Lys
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Met	Ala	Ile	Thr	Lys	Glu	His	Ser	Ile	Thr	Leu	Asn	Leu	Thr	Ile	Met
610						615					620				
Asn	Val	Ser	Leu	Gln	Asp	Ser	Gly	Thr	Tyr	Ala	Cys	Arg	Ala	Arg	Asn
625					630					635					640
Val	Tyr	Thr	Gly	Glu	Glu	Ile	Leu	Gln	Lys	Lys	Glu	Ile	Thr	Ile	Arg
			645						650					655	
Asp	Gln	Glu	Ala	Pro	Tyr	Leu	Leu	Arg	Asn	Leu	Ser	Asp	His	Thr	Val
			660					665					670		
Ala	Ile	Ser	Ser	Ser	Thr	Thr	Leu	Asp	Cys	His	Ala	Asn	Gly	Val	Pro
		675					680					685			
Glu	Pro	Gln	Ile	Thr	Trp	Phe	Lys	Asn	Asn	His	Lys	Ile	Gln	Gln	Glu
690						695					700				
Pro	Gly	Ile	Ile	Leu	Gly	Pro	Gly	Ser	Ser	Thr	Leu	Phe	Ile	Glu	Arg
705					710					715					720
Val	Thr	Glu	Glu	Asp	Glu	Gly	Val	Tyr	His	Cys	Lys	Ala	Thr	Asn	Gln
			725					730						735	
Lys	Gly	Ser	Val	Glu	Ser	Ser	Ala	Tyr	Leu	Thr	Val	Gln	Gly	Thr	Ser
			740					745					750		
Asp	Lys	Ser	Asn	Leu	Glu	Leu	Ile	Thr	Leu	Thr	Cys	Thr	Cys	Val	Ala
		755					760					765			
Ala	Thr	Leu	Phe	Trp	Leu	Leu	Leu	Thr	Leu	Phe	Ile	Arg	Lys	Met	Lys
770						775					780				
Arg	Ser	Ser	Ser	Glu	Ile	Lys	Thr	Asp	Tyr	Leu	Ser	Ile	Ile	Met	Asp
785					790					795					800
Pro	Asp	Glu	Val	Pro	Leu	Asp	Glu	Gln	Cys	Glu	Arg	Leu	Pro	Tyr	Asp
				805					810					815	
Ala	Ser	Lys	Trp	Glu	Phe	Ala	Arg	Glu	Arg	Leu	Lys	Leu	Gly	Lys	Ser
			820					825					830		
Leu	Gly	Arg	Gly	Ala	Phe	Gly	Lys	Val	Val	Gln	Ala	Ser	Ala	Phe	Gly
			835				840					845			
Ile	Lys	Lys	Ser	Pro	Thr	Cys	Arg	Thr	Val	Ala	Val	Lys	Met	Leu	Lys
850						855					860				
Glu	Gly	Ala	Thr	Ala	Ser	Glu	Tyr	Lys	Ala	Leu	Met	Thr	Glu	Leu	Lys
865					870					875					880
Ile	Leu	Thr	His	Ile	Gly	His	His	Leu	Asn	Val	Val	Asn	Leu	Leu	Gly
			885						890					895	
Ala	Cys	Thr	Lys	Gln	Gly	Gly	Pro	Leu	Met	Val	Ile	Val	Glu	Tyr	Cys
			900					905					910		
Lys	Tyr	Gly	Asn	Leu	Ser	Asn	Tyr	Leu	Lys	Ser	Lys	Arg	Asp	Leu	Phe
		915					920					925			
Phe	Leu	Asn	Lys	Asp	Ala	Ala	Leu	His	Met	Glu	Pro	Lys	Lys	Glu	Lys
930						935					940				

Met 945	Glu	Pro	Gly	Leu	Glu	Gln	Gly	Lys	Lys	Pro	Arg	Leu	Asp	Ser	Val 960
Thr	Ser	Ser	Glu	Ser	Phe	Ala	Ser	Ser	Gly	Phe	Gln	Glu	Asp	Lys	Ser 975
Leu	Ser	Asp	Val	Glu	Glu	Glu	Glu	Asp	Ser	Asp	Gly	Phe	Tyr	Lys	Glu 990
Pro	Ile	Thr	Met	Glu	Asp	Leu	Ile	Ser	Tyr	Ser	Phe	Gln	Val	Ala	Arg 1005
Gly	Met	Glu	Phe	Leu	Ser	Ser	Arg	Lys	Cys	Ile	His	Arg	Asp	Leu	
Ala	Ala	Arg	Asn	Ile	Leu	Leu	Ser	Glu	Asn	Asn	Val	Val	Lys	Ile	
Cys	Asp	Phe	Gly	Leu	Ala	Arg	Asp	Ile	Tyr	Lys	Asn	Pro	Asp	Tyr	
Val	Arg	Lys	Gly	Asp	Thr	Arg	Leu	Pro	Leu	Lys	Trp	Met	Ala	Pro	
Glu	Ser	Ile	Phe	Asp	Lys	Ile	Tyr	Ser	Thr	Lys	Ser	Asp	Val	Trp	
Ser	Tyr	Gly	Val	Leu	Leu	Trp	Glu	Ile	Phe	Ser	Leu	Gly	Gly	Ser	
Pro	Tyr	Pro	Gly	Val	Gln	Met	Asp	Glu	Asp	Phe	Cys	Ser	Arg	Leu	
Arg	Glu	Gly	Met	Arg	Met	Arg	Ala	Pro	Glu	Tyr	Ser	Thr	Pro	Glu	
Ile	Tyr	Gln	Ile	Met	Leu	Asp	Cys	Trp	His	Arg	Asp	Pro	Lys	Glu	
Arg	Pro	Arg	Phe	Ala	Glu	Leu	Val	Glu	Lys	Leu	Gly	Asp	Leu	Leu	
Gln	Ala	Asn	Val	Gln	Gln	Asp	Gly	Lys	Asp	Tyr	Ile	Pro	Ile	Asn	
Ala	Ile	Leu	Thr	Gly	Asn	Ser	Gly	Phe	Thr	Tyr	Ser	Thr	Pro	Ala	
Phe	Ser	Glu	Asp	Phe	Phe	Lys	Glu	Ser	Ile	Ser	Ala	Pro	Lys	Phe	
Asn	Ser	Gly	Ser	Ser	Asp	Asp	Val	Arg	Tyr	Val	Asn	Ala	Phe	Lys	
Phe	Met	Ser	Leu	Glu	Arg	Ile	Lys	Thr	Phe	Glu	Glu	Leu	Leu	Pro	
Asn	Ala	Thr	Ser	Met	Phe	Asp	Asp	Tyr	Gln	Gly	Asp	Ser	Ser	Thr	
Leu	Leu	Ala	Ser	Pro	Met	Leu	Lys	Arg	Phe	Thr	Trp	Thr	Asp	Ser	
Lys	Pro	Lys	Ala	Ser	Leu	Lys	Ile	Asp	Leu	Arg	Val	Thr	Ser	Lys	
Ser	Lys	Glu	Ser	Gly	Leu	Ser	Asp	Val	Ser	Arg	Pro	Ser	Phe	Cys	
His	Ser	Ser	Cys	Gly	His	Val	Ser	Glu	Gly	Lys	Arg	Arg	Phe	Thr	
Tyr	Asp	His	Ala	Glu	Leu	Glu	Arg	Lys	Ile	Ala	Cys	Cys	Ser	Pro	
Pro	Pro	Asp	Tyr	Asn	Ser	Val	Val	Leu	Tyr	Ser	Thr	Pro	Pro	Ile	

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<210> SEQ ID NO 3
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<222> LOCATION: (1)..(1356)
<223> OTHER INFORMATION: amino acid sequence of human VEGFR2

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Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr
35     40     45
Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
50     55     60
Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys Ser
65     70     75     80
Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly Asn
85     90     95
Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala Ser
100    105    110
Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro Phe Ile Ala Ser
115    120    125
Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn Lys
130    135    140
Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn Leu Asn Val Ser
145    150    155    160
Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly Asn Arg
165    170    175
Ile Ser Trp Asp Ser Lys Lys Gly Phe Thr Ile Pro Ser Tyr Met Ile
180    185    190
Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile Asn Asp Glu Ser
195    200    205
Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg Ile Tyr
210    215    220
Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
225    230    235    240
Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
245    250    255
Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
260    265    270
Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
275    280    285
Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
290    295    300
Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
305    310    315    320
Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met
325    330    335
Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg Ile Pro Ala
340    345    350
Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly
355    360    365

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Ile	Pro	Leu	Glu	Ser	Asn	His	Thr	Ile	Lys	Ala	Gly	His	Val	Leu	Thr
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Ile	Met	Glu	Val	Ser	Glu	Arg	Asp	Thr	Gly	Asn	Tyr	Thr	Val	Ile	Leu
385			390			395						400			
Thr	Asn	Pro	Ile	Ser	Lys	Glu	Lys	Gln	Ser	His	Val	Val	Ser	Leu	Val
			405			410						415			
Val	Tyr	Val	Pro	Pro	Gln	Ile	Gly	Glu	Lys	Ser	Leu	Ile	Ser	Pro	Val
			420			425						430			
Asp	Ser	Tyr	Gln	Tyr	Gly	Thr	Thr	Gln	Thr	Leu	Thr	Cys	Thr	Val	Tyr
435			440			445									
Ala	Ile	Pro	Pro	Pro	His	His	Ile	His	Trp	Tyr	Trp	Gln	Leu	Glu	Glu
450			455			460									
Glu	Cys	Ala	Asn	Glu	Pro	Ser	Gln	Ala	Val	Ser	Val	Thr	Asn	Pro	Tyr
465			470			475						480			
Pro	Cys	Glu	Glu	Trp	Arg	Ser	Val	Glu	Asp	Phe	Gln	Gly	Gly	Asn	Lys
			485			490						495			
Ile	Glu	Val	Asn	Lys	Asn	Gln	Phe	Ala	Leu	Ile	Glu	Gly	Lys	Asn	Lys
			500			505						510			
Thr	Val	Ser	Thr	Leu	Val	Ile	Gln	Ala	Ala	Asn	Val	Ser	Ala	Leu	Tyr
515			520			525									
Lys	Cys	Glu	Ala	Val	Asn	Lys	Val	Gly	Arg	Gly	Glu	Arg	Val	Ile	Ser
530			535			540									
Phe	His	Val	Thr	Arg	Gly	Pro	Glu	Ile	Thr	Leu	Gln	Pro	Asp	Met	Gln
545			550			555						560			
Pro	Thr	Glu	Gln	Glu	Ser	Val	Ser	Leu	Trp	Cys	Thr	Ala	Asp	Arg	Ser
			565			570						575			
Thr	Phe	Glu	Asn	Leu	Thr	Trp	Tyr	Lys	Leu	Gly	Pro	Gln	Pro	Leu	Pro
			580			585						590			
Ile	His	Val	Gly	Glu	Leu	Pro	Thr	Pro	Val	Cys	Lys	Asn	Leu	Asp	Thr
595			600			605									
Leu	Trp	Lys	Leu	Asn	Ala	Thr	Met	Phe	Ser	Asn	Ser	Thr	Asn	Asp	Ile
610			615			620									
Leu	Ile	Met	Glu	Leu	Lys	Asn	Ala	Ser	Leu	Gln	Asp	Gln	Gly	Asp	Tyr
625			630			635						640			
Val	Cys	Leu	Ala	Gln	Asp	Arg	Lys	Thr	Lys	Lys	Arg	His	Cys	Val	Val
			645			650						655			
Arg	Gln	Leu	Thr	Val	Leu	Glu	Arg	Val	Ala	Pro	Thr	Ile	Thr	Gly	Asn
			660			665						670			
Leu	Glu	Asn	Gln	Thr	Thr	Ser	Ile	Gly	Glu	Ser	Ile	Glu	Val	Ser	Cys
			675			680						685			
Thr	Ala	Ser	Gly	Asn	Pro	Pro	Pro	Gln	Ile	Met	Trp	Phe	Lys	Asp	Asn
690			695			700									
Glu	Thr	Leu	Val	Glu	Asp	Ser	Gly	Ile	Val	Leu	Lys	Asp	Gly	Asn	Arg
705			710			715						720			
Asn	Leu	Thr	Ile	Arg	Arg	Val	Arg	Lys	Glu	Asp	Glu	Gly	Leu	Tyr	Thr
			725			730						735			
Cys	Gln	Ala	Cys	Ser	Val	Leu	Gly	Cys	Ala	Lys	Val	Glu	Ala	Phe	Phe
740			745			750									
Ile	Ile	Glu	Gly	Ala	Gln	Glu	Lys	Thr	Asn	Leu	Glu	Ile	Ile	Ile	Leu
755			760			765									
Val	Gly	Thr	Ala	Val	Ile	Ala	Met	Phe	Phe	Trp	Leu	Leu	Leu	Val	Ile
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<210> SEQ ID NO 6
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
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<223> OTHER INFORMATION: nucleotide sequence of human VEGFR2 (CDS:
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<400> SEQUENCE: 6

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<210> SEQ ID NO 7
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 <220> FEATURE:
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 <222> LOCATION: (1)..(223)
 <223> OTHER INFORMATION: amino acid sequence of human IgG Fc

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20          25          30

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
35          40          45

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
50          55          60

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
65          70          75          80

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
85          90          95

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
100         105         110

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
115         120         125

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
130         135         140

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
145         150         155         160

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
165         170         175

Asp Gly Pro Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
180         185         190

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
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His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
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<210> SEQ ID NO 8
 <211> LENGTH: 669
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(669)
 <223> OTHER INFORMATION: DNA sequence of human IgG FC

<400> SEQUENCE: 8

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<210> SEQ ID NO 9
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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: #19 fusion protein

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<400> SEQUENCE: 9

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Arg Cys Arg Leu Arg Asp Asp Val Gln Ser Ile Asn Trp Leu Arg Asp
35         40         45
Gly Val Gln Leu Ala Glu Ser Asn Arg Thr Arg Ile Thr Gly Glu Glu
50         55         60
Val Glu Val Gln Asp Ser Val Pro Ala Asp Ser Gly Leu Tyr Ala Cys
65         70         75         80
Val Thr Ser Ser Pro Ser Gly Ser Asp Thr Thr Tyr Phe Ser Val Asn
85         90         95
Val Ser Asp Ala Leu Pro Ser Ser Glu Asp Asp Asp Asp Asp Asp
100        105        110
Ser Ser Ser Glu Glu Lys Glu Thr Asp Asn Thr Lys Pro Asn Pro Val
115        120        125
Ala Pro Tyr Trp Thr Ser Pro Glu Lys Met Glu Lys Lys Leu His Ala
130        135        140
Val Pro Ala Ala Lys Thr Val Lys Phe Lys Cys Pro Ser Ser Gly Thr
145        150        155        160
Pro Asn Pro Thr Leu Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro
165        170        175
Asp His Arg Ile Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile
180        185        190
Ile Met Asp Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile
195        200        205
Val Glu Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr Gln Leu Asp Val
210        215        220
Val Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala
225        230        235        240
Asn Lys Thr Val Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val
245        250        255
Tyr Ser Asp Pro Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val
260        265        270
Asn Gly Ser Lys Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu
275        280        285
Lys Thr Ala Gly Val Asn Thr Thr Asp Lys Glu Met Glu Val Leu His
290        295        300

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Glu Ala Leu Glu Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Leu
                      340          345          350

Glu Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
                      355          360          365

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
370          375          380

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
385          390          395          400

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
                      405          410          415

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
                      420          425          430

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
435          440          445

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
450          455          460

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
465          470          475          480

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
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Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
500          505          510

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
515          520          525

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
530          535          540

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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Ser Pro Gly Lys
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<210> SEQ ID NO 10

<211> LENGTH: 526

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: #13 fusion protein

<400> SEQUENCE: 10

```

Lys Asn Arg Thr Arg Ile Thr Gly Glu Glu Val Glu Val Gln Asp Ser
1                      5                      10          15

Val Pro Ala Asp Ser Gly Leu Tyr Ala Cys Val Thr Ser Ser Pro Ser
20          25          30

Gly Ser Asp Thr Thr Tyr Phe Ser Val Asn Val Ser Asp Ala Leu Pro
35          40          45

Ser Ser Glu Asp Asp Asp Asp Asp Asp Ser Ser Ser Glu Glu Lys
50          55          60

Glu Thr Asp Asn Thr Lys Pro Asn Pro Val Ala Pro Tyr Trp Thr Ser
65          70          75          80

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Pro 85	Glu 85	Lys 85	Met 85	Glu 85	Lys 85	Lys 85	Leu 85	His 90	Ala 90	Val 90	Pro 90	Ala 90	Ala 95	Lys 95	Thr 95
Val 100	Lys 100	Phe 100	Lys 100	Cys 100	Pro 100	Ser 100	Ser 100	Gly 105	Thr 105	Pro 105	Asn 105	Pro 110	Thr 110	Leu 110	Arg 110
Trp 115	Leu 115	Lys 115	Asn 115	Gly 115	Lys 115	Glu 115	Phe 120	Lys 120	Pro 120	Asp 120	His 125	Arg 125	Ile 125	Gly 125	Gly 125
Tyr 130	Lys 130	Val 130	Arg 130	Tyr 130	Ala 130	Thr 135	Trp 135	Ser 135	Ile 135	Ile 140	Met 140	Asp 140	Ser 140	Val 140	Val 140
Pro 145	Ser 145	Asp 145	Lys 145	Gly 145	Asn 150	Tyr 150	Thr 150	Cys 150	Ile 150	Val 155	Glu 155	Asn 155	Glu 155	Tyr 160	Gly 160
Ser 165	Ile 165	Asn 165	His 165	Thr 165	Tyr 165	Gln 165	Leu 165	Asp 170	Val 170	Val 170	Glu 170	Arg 170	Ser 175	Pro 175	His 175
Arg 180	Pro 180	Ile 180	Leu 180	Gln 180	Ala 180	Gly 180	Leu 185	Pro 185	Ala 185	Asn 185	Lys 185	Thr 185	Val 190	Ala 190	Leu 190
Gly 195	Ser 195	Asn 195	Val 195	Glu 195	Phe 195	Met 195	Cys 200	Lys 200	Val 200	Tyr 200	Ser 205	Asp 205	Pro 205	Gln 205	Pro 205
His 210	Ile 210	Gln 210	Trp 210	Leu 210	Lys 215	His 215	Ile 215	Glu 215	Val 215	Asn 220	Gly 220	Ser 220	Lys 220	Ile 220	Gly 220
Pro 225	Asp 225	Asn 225	Leu 225	Pro 225	Tyr 230	Val 230	Gln 230	Ile 230	Leu 235	Lys 235	Thr 235	Ala 235	Gly 235	Val 240	Asn 240
Thr 245	Thr 245	Asp 245	Lys 245	Glu 245	Met 245	Glu 245	Val 245	Leu 250	His 250	Leu 250	Arg 250	Asn 255	Val 255	Ser 255	Phe 255
Glu 260	Asp 260	Ala 260	Gly 260	Glu 260	Tyr 260	Thr 260	Cys 265	Leu 265	Ala 265	Gly 265	Asn 265	Ser 270	Ile 270	Gly 270	Leu 270
Ser 275	His 275	His 275	Ser 275	Ala 275	Trp 275	Leu 275	Thr 280	Val 280	Leu 280	Glu 280	Ala 285	Leu 285	Glu 285	Glu 285	Arg 285
Pro 290	Ala 290	Val 290	Met 290	Thr 290	Ser 295	Pro 295	Leu 295	Tyr 295	Leu 295	Glu 300	Asp 300	Lys 300	Thr 300	His 300	Thr 300
Cys 305	Pro 305	Pro 305	Cys 305	Pro 305	Ala 310	Pro 310	Glu 310	Leu 310	Leu 315	Gly 315	Gly 315	Pro 315	Ser 315	Val 315	Phe 320
Leu 325	Phe 325	Pro 325	Pro 325	Lys 325	Pro 325	Lys 325	Asp 325	Thr 330	Leu 330	Met 330	Ile 330	Ser 330	Arg 335	Thr 335	Pro 335
Glu 340	Val 340	Thr 340	Cys 340	Val 340	Val 340	Val 340	Asp 345	Val 345	Ser 345	His 345	Glu 345	Asp 350	Pro 350	Glu 350	Val 350
Lys 355	Phe 355	Asn 355	Trp 355	Tyr 355	Val 355	Asp 355	Gly 360	Val 360	Glu 360	Val 360	His 365	Asn 365	Ala 365	Lys 365	Thr 365
Lys 370	Pro 370	Arg 370	Glu 370	Glu 370	Gln 375	Tyr 375	Asn 375	Ser 375	Thr 375	Tyr 380	Arg 380	Val 380	Val 380	Ser 380	Val 380
Leu 385	Thr 385	Val 385	Leu 385	His 385	Gln 390	Asp 390	Trp 390	Leu 390	Asn 395	Gly 395	Lys 395	Glu 395	Tyr 395	Lys 400	Cys 400
Lys 405	Val 405	Ser 405	Asn 405	Lys 405	Ala 405	Leu 405	Pro 410	Ala 410	Pro 410	Ile 410	Glu 410	Lys 415	Thr 415	Ile 415	Ser 415
Lys 420	Ala 420	Lys 420	Gly 420	Gln 420	Pro 420	Arg 420	Glu 425	Pro 425	Gln 425	Val 425	Tyr 425	Thr 430	Leu 430	Pro 430	Pro 430
Ser 435	Arg 435	Asp 435	Glu 435	Leu 435	Thr 435	Lys 440	Asn 440	Gln 440	Val 440	Ser 440	Leu 445	Thr 445	Cys 445	Leu 445	Val 445
Lys 450	Gly 450	Phe 450	Tyr 450	Pro 450	Ser 455	Asp 455	Ile 455	Ala 455	Val 455	Glu 460	Trp 460	Glu 460	Ser 460	Asn 460	Gly 460
Gln 465	Pro 465	Glu 465	Asn 465	Asn 465	Tyr 470	Lys 470	Thr 470</								

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500	505	510
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		
515	520	525
<210> SEQ ID NO 11		
<211> LENGTH: 500		
<212> TYPE: PRT		
<213> ORGANISM: Artificial sequence		
<220> FEATURE:		
<223> OTHER INFORMATION: #22 fusion protein		
<400> SEQUENCE: 11		
Val Thr Ser Ser Pro Ser Gly Ser Asp Thr Thr Tyr Phe Ser Val Asn		
1	5	10
Val Ser Asp Ala Leu Pro Ser Ser Glu Asp Asp Asp Asp Asp Asp Asp		
20	25	30
Ser Ser Ser Glu Glu Lys Glu Thr Asp Asn Thr Lys Pro Asn Pro Val		
35	40	45
Ala Pro Tyr Trp Thr Ser Pro Glu Lys Met Glu Lys Lys Leu His Ala		
50	55	60
Val Pro Ala Ala Lys Thr Val Lys Phe Lys Cys Pro Ser Ser Gly Thr		
65	70	75
Pro Asn Pro Thr Leu Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro		
85	90	95
Asp His Arg Ile Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile		
100	105	110
Ile Met Asp Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile		
115	120	125
Val Glu Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr Gln Leu Asp Val		
130	135	140
Val Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala		
145	150	155
Asn Lys Thr Val Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val		
165	170	175
Tyr Ser Asp Pro Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val		
180	185	190
Asn Gly Ser Lys Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu		
195	200	205
Lys Thr Ala Gly Val Asn Thr Thr Asp Lys Glu Met Glu Val Leu His		
210	215	220
Leu Arg Asn Val Ser Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala		
225	230	235
Gly Asn Ser Ile Gly Leu Ser His His Ser Ala Trp Leu Thr Val Leu		
245	250	255
Glu Ala Leu Glu Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Leu		
260	265	270
Glu Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu		
275	280	285
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		
290	295	300
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
305	310	315
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		
325	330	335
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		

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340	345	350
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
355	360	365
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
370	375	380
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln		
385	390	395
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val		
405	410	415
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
420	425	430
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
435	440	445
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
450	455	460
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
465	470	475
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
485	490	495
Ser Pro Gly Lys		
500		

<210> SEQ ID NO 12
 <211> LENGTH: 468
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: #23 fusion protein

<400> SEQUENCE: 12

Ser Ser Ser Glu Glu Lys Glu Thr Asp Asn Thr Lys Pro Asn Pro Val		
1	5	10
Ala Pro Tyr Trp Thr Ser Pro Glu Lys Met Glu Lys Lys Leu His Ala		
20	25	30
Val Pro Ala Ala Lys Thr Val Lys Phe Lys Cys Pro Ser Ser Gly Thr		
35	40	45
Pro Asn Pro Thr Leu Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro		
50	55	60
Asp His Arg Ile Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile		
65	70	75
Ile Met Asp Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile		
85	90	95
Val Glu Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr Gln Leu Asp Val		
100	105	110
Val Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala		
115	120	125
Asn Lys Thr Val Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val		
130	135	140
Tyr Ser Asp Pro Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val		
145	150	155
Asn Gly Ser Lys Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu		
165	170	175
Lys Thr Ala Gly Val Asn Thr Thr Asp Lys Glu Met Glu Val Leu His		
180	185	190
Leu Arg Asn Val Ser Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala		

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195	200	205
Gly Asn Ser Ile Gly Leu	Ser His His Ser Ala Trp	Leu Thr Val Leu
210	215	220
Glu Ala Leu Glu Glu Arg	Pro Ala Val Met Thr Ser	Pro Leu Tyr Leu
225	230	235 240
Glu Asp Lys Thr His Thr	Cys Pro Pro Cys Pro Ala	Pro Glu Leu Leu
	245	250 255
Gly Gly Pro Ser Val Phe	Leu Phe Pro Pro Lys Pro	Lys Asp Thr Leu
	260	265 270
Met Ile Ser Arg Thr Pro	Glu Val Thr Cys Val Val	Val Asp Val Ser
	275	280 285
His Glu Asp Pro Glu Val	Lys Phe Asn Trp Tyr Val	Asp Gly Val Glu
	290	295 300
Val His Asn Ala Lys Thr	Lys Pro Arg Glu Glu Gln	Tyr Asn Ser Thr
	305	310 315 320
Tyr Arg Val Val Ser Val	Leu Thr Val Leu His Gln	Asp Trp Leu Asn
	325	330 335
Gly Lys Glu Tyr Lys Cys	Lys Val Ser Asn Lys Ala	Leu Pro Ala Pro
	340	345 350
Ile Glu Lys Thr Ile Ser	Lys Ala Lys Gly Gln Pro	Arg Glu Pro Gln
	355	360 365
Val Tyr Thr Leu Pro Pro	Ser Arg Asp Glu Leu Thr	Lys Asn Gln Val
	370	375 380
Ser Leu Thr Cys Leu Val	Lys Gly Phe Tyr Pro Ser	Asp Ile Ala Val
	385	390 395 400
Glu Trp Glu Ser Asn Gly	Gln Pro Glu Asn Asn Tyr	Lys Thr Thr Pro
	405	410 415
Pro Val Leu Asp Ser Asp	Gly Ser Phe Phe Leu Tyr	Ser Lys Leu Thr
	420	425 430
Val Asp Lys Ser Arg Trp	Gln Gln Gly Asn Val Phe	Ser Cys Ser Val
	435	440 445
Met His Glu Ala Leu His	Asn His Tyr Thr Gln Lys	Ser Leu Ser Leu
	450	455 460
Ser Pro Gly Lys		
465		

<210> SEQ ID NO 13

<211> LENGTH: 457

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: #26 fusion protein

<400> SEQUENCE: 13

Lys Pro Asn Pro Val Ala	Pro Tyr Trp Thr Ser	Pro Glu Lys Met Glu
1	5	10 15
Lys Lys Leu His Ala Val	Pro Ala Ala Lys Thr	Val Lys Phe Lys Cys
	20	25 30
Pro Ser Ser Gly Thr Pro	Asn Pro Thr Leu Arg Trp	Leu Lys Asn Gly
	35	40 45
Lys Glu Phe Lys Pro Asp	His Arg Ile Gly Gly Tyr	Lys Val Arg Tyr
	50	55 60
Ala Thr Trp Ser Ile Ile	Met Asp Ser Val Val	Pro Ser Asp Lys Gly
	65	70 75 80
Asn Tyr Thr Cys Ile Val	Glu Asn Glu Tyr Gly	Ser Ile Asn His Thr

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85					90					95					
Tyr	Gln	Leu	Asp	Val	Val	Glu	Arg	Ser	Pro	His	Arg	Pro	Ile	Leu	Gln
			100					105					110		
Ala	Gly	Leu	Pro	Ala	Asn	Lys	Thr	Val	Ala	Leu	Gly	Ser	Asn	Val	Glu
		115					120					125			
Phe	Met	Cys	Lys	Val	Tyr	Ser	Asp	Pro	Gln	Pro	His	Ile	Gln	Trp	Leu
		130					135					140			
Lys	His	Ile	Glu	Val	Asn	Gly	Ser	Lys	Ile	Gly	Pro	Asp	Asn	Leu	Pro
						150					155				160
Tyr	Val	Gln	Ile	Leu	Lys	Thr	Ala	Gly	Val	Asn	Thr	Thr	Asp	Lys	Glu
				165					170					175	
Met	Glu	Val	Leu	His	Leu	Arg	Asn	Val	Ser	Phe	Glu	Asp	Ala	Gly	Glu
			180					185					190		
Tyr	Thr	Cys	Leu	Ala	Gly	Asn	Ser	Ile	Gly	Leu	Ser	His	His	Ser	Ala
			195				200					205			
Trp	Leu	Thr	Val	Leu	Glu	Ala	Leu	Glu	Glu	Arg	Pro	Ala	Val	Met	Thr
			210				215					220			
Ser	Pro	Leu	Tyr	Leu	Glu	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro
				230							235				240
Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys
				245					250					255	
Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val
			260					265					270		
Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr
			275					280					285		
Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu
		290					295					300			
Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His
				310							315				320
Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys
				325					330					335	
Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln
				340				345					350		
Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu
			355					360					365		
Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro
				370				375				380			
Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn
				385					390			395			400
Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu
				405					410					415	
Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val
			420					425					430		
Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln
				435				440					445		
Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys							
			450					455							

<210> SEQ ID NO 14

<211> LENGTH: 451

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: #29 fusion protein

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<400> SEQUENCE: 14

Pro Tyr Trp Thr Ser Pro Glu Lys Met Glu Lys Lys Leu His Ala Val
 1 5 10 15
 Pro Ala Ala Lys Thr Val Lys Phe Lys Cys Pro Ser Ser Gly Thr Pro
 20 25 30
 Asn Pro Thr Leu Arg Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro Asp
 35 40 45
 His Arg Ile Gly Gly Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile Ile
 50 55 60
 Met Asp Ser Val Val Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile Val
 65 70 75 80
 Glu Asn Glu Tyr Gly Ser Ile Asn His Thr Tyr Gln Leu Asp Val Val
 85 90 95
 Glu Arg Ser Pro His Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn
 100 105 110
 Lys Thr Val Ala Leu Gly Ser Asn Val Glu Phe Met Cys Lys Val Tyr
 115 120 125
 Ser Asp Pro Gln Pro His Ile Gln Trp Leu Lys His Ile Glu Val Asn
 130 135 140
 Gly Ser Lys Ile Gly Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu Lys
 145 150 155 160
 Thr Ala Gly Val Asn Thr Thr Asp Lys Glu Met Glu Val Leu His Leu
 165 170 175
 Arg Asn Val Ser Phe Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly
 180 185 190
 Asn Ser Ile Gly Leu Ser His His Ser Ala Trp Leu Thr Val Leu Glu
 195 200 205
 Ala Leu Glu Glu Arg Pro Ala Val Met Thr Ser Pro Leu Tyr Leu Glu
 210 215 220
 Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
 225 230 235 240
 Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
 245 250 255
 Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
 260 265 270
 Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
 275 280 285
 His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
 290 295 300
 Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
 305 310 315 320
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
 325 330 335
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
 340 345 350
 Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
 355 360 365
 Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 370 375 380
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
 385 390 395 400
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
 405 410 415

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Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Lys
450

<210> SEQ ID NO 15

<211> LENGTH: 446

<212> TYPE: PRT

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: #8 fusion protein

<400> SEQUENCE: 15

Pro Glu Lys Met Glu Lys Lys Leu His Ala Val Pro Ala Ala Lys Thr
1 5 10 15

Val Lys Phe Lys Cys Pro Ser Ser Gly Thr Pro Asn Pro Thr Leu Arg
20 25 30

Trp Leu Lys Asn Gly Lys Glu Phe Lys Pro Asp His Arg Ile Gly Gly
35 40 45

Tyr Lys Val Arg Tyr Ala Thr Trp Ser Ile Ile Met Asp Ser Val Val
50 55 60

Pro Ser Asp Lys Gly Asn Tyr Thr Cys Ile Val Glu Asn Glu Tyr Gly
65 70 75 80

Ser Ile Asn His Thr Tyr Gln Leu Asp Val Val Glu Arg Ser Pro His
85 90 95

Arg Pro Ile Leu Gln Ala Gly Leu Pro Ala Asn Lys Thr Val Ala Leu
100 105 110

Gly Ser Asn Val Glu Phe Met Cys Lys Val Tyr Ser Asp Pro Gln Pro
115 120 125

His Ile Gln Trp Leu Lys His Ile Glu Val Asn Gly Ser Lys Ile Gly
130 135 140

Pro Asp Asn Leu Pro Tyr Val Gln Ile Leu Lys Thr Ala Gly Val Asn
145 150 155 160

Thr Thr Asp Lys Glu Met Glu Val Leu His Leu Arg Asn Val Ser Phe
165 170 175

Glu Asp Ala Gly Glu Tyr Thr Cys Leu Ala Gly Asn Ser Ile Gly Leu
180 185 190

Ser His His Ser Ala Trp Leu Thr Val Leu Glu Ala Leu Glu Glu Arg
195 200 205

Pro Ala Val Met Thr Ser Pro Leu Tyr Leu Glu Asp Lys Thr His Thr
210 215 220

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
225 230 235 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
245 250 255

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
260 265 270

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
275 280 285

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
290 295 300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305 310 315 320

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Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
 325 330 335
 Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
 340 345 350
 Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
 355 360 365
 Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
 370 375 380
 Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
 385 390 395 400
 Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
 405 410 415
 Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
 420 425 430
 Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440 445

<210> SEQ ID NO 16
 <211> LENGTH: 1743
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: nucleotide sequence of #19 fusion protein

<400> SEQUENCE: 16

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aggccgtccc cgaccttgcc tgaacaagcc cagccctggg gagccctgt ggaagtggag      60
tccttctctgg tccaccccg tgacctgcta cagcttcgct gtcggctgcg ggacgatgtg      120
cagagcatca actggctgcg ggacgggggtg cagctggcgg aaagcaatcg caccgcacac      180
acaggggagg aggtggagggt gcaggactcc gtgcccgag actccggcct ctatgcttgc      240
gtaaccagca gcccttcggg cagtgcacac acctacttct cgtcaatgt ttcagatgct      300
ctccctctct cggaggatga tgatgatgat gatgactcct cttcagagga gaaagaaaca      360
gataacacca aacaaacccc cgtagctcca tattggacat cccagaaaa gatggaaaag      420
aaattgcatg cagtgcgggc tgccaagaca gtgaagttca aatgcccttc cagtgggacc      480
ccaaacccca cactgcgctg gttgaaaaat ggcaaagaat tcaaacctga ccacagaatt      540
ggaggtctaca aggtccgtta tgccacctgg agcatcataa tggactctgt ggtgccctct      600
gacaagggca actacacctg cattgtggag aatgagtacg gcagcatcaa ccacacatac      660
cagctggatg tcgtggagcg gtccctcac cggcccatcc tgcaagcagg gttgcccgcc      720
aacaaaacag tggccctggg tagcaacgtg gagttcatgt gtaagggtga cagtgacccg      780
cagccgcaca tccagtggct aaagcacatc gaggtgaatg ggagcaagat tggccagac      840
aacctgcctt atgtccagat cttgaagact gctggagtta ataccaccga caaagagatg      900
gaggtgcttc acttaagaaa tgtctccttt gaggacgcag gggagtatac gtgcttggcg      960
ggtaactcta tcggactctc ccatcactct gcatggttga ccgttctgga agccctggaa     1020
gagaggccgg cagtgatgac ctgcacctg tacctggagg aaaaaactca cacatgccca     1080
ccgtgccag cacctgaact cctgggggga ccgtcagtct tctcttccc cccaaaaacc     1140
aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtgggtgt ggacgtgagc     1200
cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc     1260
aagacaaaag cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc     1320

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gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggctctc caacaaagcc	1380
ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag	1440
gtgtacaccc tgccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc	1500
ctggtcaaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg	1560
gagaacaact acaagaccac gcctcccgctg ctggactccg acggctcctt ctctctctac	1620
agcaagctca ccgtggacaa gaggaggtgg cagcagggga acgtctcttc atgctccgtg	1680
atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaaa	1740
tga	1743

<210> SEQ ID NO 17
 <211> LENGTH: 1581
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: nucleotide sequence of #13 fusion protein

<400> SEQUENCE: 17

aaaaatcgca cccgcatcac aggggaggag gtggagggtc aggactccgt gccgcagac	60
tccggcctct atgcttgctg aaccagcagc cctcgggca gtgacaccac ctactctctc	120
gtcaatgttt cagatgtctc cccctcctcg gaggatgatg atgatgatga tgactcctct	180
tcagaggaga aagaacaga taacacccaa ccaaaccccg tagctccata ttggacatcc	240
ccagaaaaga tggaaaagaa attgcatgca gtgcggctg ccaagacagt gaagtcaaa	300
tgcccttcca gtgggacccc aaaccacaca ctgcgtggt tgaaaaatgg caaagaattc	360
aaactgacc acagaattgg aggtctacaag gtccgttatg ccacctggag catcataatg	420
gactctgtgg tgccctctga caagggaac tacacctgca ttgtggagaa tgagtacggc	480
agcatcaacc acacatacca gctggatgtc gtggagcggc cccctcaccg gccatcctg	540
caagcagggt tgcccgcaca caaacagtg gccctgggta gcaacgtgga gttcatgtgt	600
aagggtgtaca gtgaccgca gccgcacatc cagtggctaa agcacatcga ggtgaatggg	660
agcaagattg gccagacaa cctgccttat gtccagatct tgaagactgc tggagttaat	720
accaccgaca aagagatgga ggtgcttcac ttaagaaatg tctcctttga ggacgcaggg	780
gagtatacgt gcttggcggg taactctatc ggactctccc atcactctgc atggttgacc	840
gttctggaag ccctggaaga gaggccggca gtgatgacct cgcctctgta cctggaggac	900
aaaactcaca catgccacc gtgcccagca cctgaactcc tggggggacc gtcagtcttc	960
ctcttccccc caaaacccaa ggacacctc atgatctccc ggacctctga ggtcacatgc	1020
gtggtggtgg acgtgagcca cgaagacct gaggtcaagt tcaactggta cgtggacggc	1080
gtggagggtc ataatgcaa gacaaagccg cgggaggagc agtacaacag cactgacctg	1140
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aaggctctcca acaaagccct ccagccccc atcgagaaaa ccatctccaa agccaaaggg	1260
cagccccgag aaccacaggt gtacacctg ccccatccc gggatgagct gaccaagaac	1320
caggtcagcc tgacctgctt ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg	1380
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac	1440
ggctcctctt tcctctacag caagctcacc gtggacaaga gcagggtggc gcaggggaac	1500
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc	1560
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<210> SEQ ID NO 18
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 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: nucleotide sequence of #22 fusion protein

<400> SEQUENCE: 18

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gataacacca aaccaaacc cgtagctcca tattggacat cccagaaaa gatggaaaag      180
aatgtgatg cagtgcgggc tgccaagaca gtgaagtcca aatgcccttc cagtgggacc      240
ccaaacccca cactgcgctg gttgaaaaat ggcaagaat tcaaacctga ccacagaatt      300
ggaggctaca aggtccgcta tgccacctgg agcatcataa tggactctgt ggtgccctct      360
gacaagggca actacacctg cattgtggag aatgagtacg gcagcatcaa ccacacatac      420
cagctggatg tcgtggagcg gtccctcac cggtccatcc tgcaagcagg gttgcccgcc      480
aacaaaacag tggccctggg tagcaacgtg gagttcatgt gtaagggtga cagtgacctg      540
cagccgcaca tccagtggct aaagcacatc gaggtgaatg ggagcaagat tggcccagac      600
aacctgcctt atgtccagat cttgaagact gctggagtta ataccaccga caaagagatg      660
gagggtgctt acttaagaaa tgtctccttt gaggacgcag gggagtatac gtgcttggcg      720
ggtaactcta tcggactctc ccatactctt gcatggttga ccgttctgga agccctggaa      780
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tga                                                                 1503

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<210> SEQ ID NO 19
 <211> LENGTH: 1407
 <212> TYPE: DNA
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: nucleotide sequence of #23 fusion protein

<400> SEQUENCE: 19

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tcctcttcag aggagaaaga aacagataac accaaaccaa acccctagc tccatattgg      60
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ttcaaatgcc cttccagtgg gaccccaaac cccacactgc gctgggtgaa aaatggcaaa      180

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gaattcaaac ctgaccacag aattggaggc tacaagggtcc gttatgccac ctggagcatc	240
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tacggcagca tcaaccacac ataccagctg gatgtcgtgg agcgggtccc tcaccggccc	360
atcctgcaag cagggttgcc cgccaacaaa acagtggccc tgggtagcaa cgtggagtgc	420
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aatgggagca agattggccc agacaacctg ccttatgtcc agatcttgaa gactgctgga	540
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gcaggggagt atacgtgctt ggcggttaac tctatcgac tctcccatca ctctgcatgg	660
ttgaccgttc tggaagccct ggaagagagg ccggcagtga tgacctcgcc cctgtacctg	720
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taccgtgtgg tcagcgtcct caccgtcctg caccaggact ggctgaatgg caaggagtac	1020
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tccgacggct ccttcttctc ctacagcaag ctcaccgtgg acaagagcag gtggcagcag	1320
gggaacgtct tctcatgtc cgtgatgcat gaggtctctg acaaccacta cagcagaag	1380
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<210> SEQ ID NO 20

<211> LENGTH: 1374

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: nucleotide sequence of #26 fusion protein

<400> SEQUENCE: 20

aaacctaac cctgagctcc atattggaca tccccagaaa agatggaaaa gaaattgcat	60
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acactgcgct ggttgaaaaa tggcaagaa ttcaaacctg accacagaat tggaggctac	180
aaggctcggt atgccacctg gagcatcata atggactctg tgggtgccctc tgacaagggc	240
aactacacct gcattgtgga gaatgagtac ggcagcatca accacacata ccagctggat	300
gtcgtggagc ggtccccca ccggcccatc ctgcaagcag ggttgccccg caacaaaaca	360
gtggccctgg gtacgaacgt ggagttcatg tgtaaggtgt acagtgacct gcagccgcac	420
atccagtggc taaagcacat cgagggtaat gggagcaaga ttggcccaga caacctgcct	480
tatgtccaga tcttgaagac tgctggagtt aataccaccg acaagagat ggaggtgctt	540
cacttaagaa atgtctcctt tgaggacgca ggggagtata cgtgcttggc gggtaactct	600
atcgactct cccatcactc tgcattggtg accgttctgg aagccctgga agagaggccg	660
gcagtgatga cctgcacct gtacctggag gacaaaactc acacatgcc accgtgccc	720
gcacctgaac tcctgggggg accgtcagtc ttcctcttcc ccccaaaacc caaggacacc	780
ctcatgatct cccggacccc tgaggtcaca tgcgtggtgg tggacgtgag ccacgaagac	840

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cctgagggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag    900
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caggactggc tgaatggcaa ggagtacaag tgcaagggtct ccaacaaagc cctcccagcc    1020
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accgtggaca agagcagggt gcagcagggg aacgtcttct catgctccgt gatgcatgag    1320
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<210> SEQ ID NO 21
<211> LENGTH: 1356
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleotide sequence of #29 fusion protein

<400> SEQUENCE: 21

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aatggcaaag aattcaaacc tgaccacaga attggaggct acaagggtccg ttatgccacc    180
tggagcatca taatggactc tgtggtgccc tctgacaagg gcaactacac ctgcattgtg    240
gagaatgagt acggcagcat caaccacaca taccagctgg atgtcgtgga gcgggtcccct    300
caccggccca tcctgcaagc aggggtgccc gccaaacaaa cagtggccct gggtagcaac    360
gtggagtcca tgtgtaagggt gtacagtgc cgcagccgc acatccagtg gctaaagcac    420
atcgagggtga atggggagcaa gattggccca gacaacctgc cttatgtcca gatcttgaa    480
actgctggag ttaataccac cgacaagag atggagggtgc ttcaactaag aaatgtctcc    540
tttgaggacg caggggagta tacgtgcttg gcgggtaact ctatcgact ctcccatcac    600
tctgcatggt tgaccgttct ggaagccctg gaagagaggg cggcagtgat gacctcgccc    660
ctgtacctgg aggacaaaac tcacacatgc ccaccgtgcc cagcacctga actcctgggg    720
ggaccgtcag tcttctctct ccccccaaaa cccaaggaca ccctcatgat ctcccgacc    780
cctgagggtca catgcgtggt ggtggacgtg agccacgaag accctgaggt caagttcaac    840
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aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc    960
aaggagtaca agtgcaaggt ctccaacaaa gccctcccag ccccatcga gaaaaccatc    1020
tccaaagcca aagggcagcc ccgagaacca caggtgtaca ccctgcccc atcccgggat    1080
gagctgacca agaaccaggt cagcctgacc tgcctgggtc aaggcttcta tcccagcgac    1140
atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc    1200
gtgctggact ccgacggctc cttcttctc tacagcaagc tcaccgtgga caagagcagg    1260
tggcagcagg ggaacgtctt ctcatgtcc gtgatgcatg aggtctctga caaccactac    1320
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<210> SEQ ID NO 22
<211> LENGTH: 1341
<212> TYPE: DNA

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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleotide sequence of #8 fusion protein

<400> SEQUENCE: 22

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tgcccttcca gtgggacccc aaaccccaca ctgcgctggt tgaaaaatgg caaagaattc    120
aaacctgacc acagaattgg aggctacaag gtccgttatg ccacctggag catcataatg    180
gactctgtgg tgccctctga caagggaac tacacctgca ttgtggagaa tgagtacggc    240
agcatcaacc acacatacca gctggatgtc gtggagcggt cccctcacgg gccatcctg    300
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agcaagattg gccagacaa cctgccttat gtccagatct tgaagactgc tggagttaat    480
accaccgaca aagagatgga ggtgcttcac ttaagaaatg tctcctttga ggacgcaggg    540
gagtatacgt gcttggcggg taactctatc ggactctccc atcactctgc atggttgacc    600
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aaaactcaca catgccacc gtgccagca cctgaactcc tgggggggacc gtcagtcttc    720
ctcttcccc caaaacccaa ggacacctc atgatctccc ggacctctga ggtcacatgc    780
gtggtggtgg acgtgagcca cgaagacct gaggtcaagt tcaactggta cgtggacggc    840
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gtggtcagcg tcctcacctg cctgcaccag gactggctga atggcaagga gtacaagtgc    960
aagggtctcca acaaaagcct ccagacccc atcgagaaaa ccatctccaa agccaaaggg    1020
cagccccgag aaccacaggt gtacacctg ccccatccc gggatgagct gaccaagaac    1080
caggtcagcc tgacctgctt ggtcaaagc ttctatccca gcgacatcgc cgtggagtgg    1140
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ggctccttct tcctctacag caagctcacc gtggacaaga gcagggtggc gcagggggaa    1260
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc    1320
tccctgtctc cgggtaaatg a                                         1341

<210> SEQ ID NO 23
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(78)
<223> OTHER INFORMATION: DNA sequence of VEGFR1 signal peptide

<400> SEQUENCE: 23

atggtcagct actgggacac cggggctctg ctgtgcgcgc tgctcagctg tctgcttctc    60

acaggatcta gttccgga                                         78

<210> SEQ ID NO 24
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: forward primer DNA sequence of #19 fusion
protein

<400> SEQUENCE: 24

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tagttccgga aggccgtccc cgaccttgcc tg 32

<210> SEQ ID NO 25
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<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: forward primer of DNA sequence of #13 fusion protein

<400> SEQUENCE: 25

tagttccgga aaaaatcgca cccgcatcac ag 32

<210> SEQ ID NO 26
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: forward primer DNA sequence of #22 fusion protein

<400> SEQUENCE: 26

tagttccgga gtaaccagca gcccctcggg c 31

<210> SEQ ID NO 27
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: forward primer DNA sequence of #23 fusion protein

<400> SEQUENCE: 27

tagttccgga tcctcttcag aggagaaaga aac 33

<210> SEQ ID NO 28
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: forward primer DNA sequence of #26 fusion protein

<400> SEQUENCE: 28

tagttccgga aaacctaacc ccgtagctcc at 32

<210> SEQ ID NO 29
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: forward primer DNA sequence of #29 fusion protein

<400> SEQUENCE: 29

tagttccgga ccatattgga catccccaga aaag 34

<210> SEQ ID NO 30
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: forward primer DNA sequence of #8 fusion protein

<400> SEQUENCE: 30

ctagctccgg accagaaaag atggaaaaga aattgc 36

-continued

<210> SEQ ID NO 31
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: reverse primer DNA sequence of FGFR1

<400> SEQUENCE: 31

gttttgcct ccaggtacag gggcgaggtc 30

<210> SEQ ID NO 32
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: forward primer DNA sequence of human IgG Fc

<400> SEQUENCE: 32

ctgtacctgg aggacaaaac tcacacatgc 30

<210> SEQ ID NO 33
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: reverse primer DNA sequence of human IgG Fc

<400> SEQUENCE: 33

gatatctgca gtcatttacc cggagacagg 30

- We claim:
1. An isolated soluble fusion protein comprising SEQ ID NO: 12.

2. An isolated soluble fusion protein comprising SEQ ID NO: 13.

3. An isolated soluble fusion protein comprising SEQ ID NO: 14.

4. The isolated soluble fusion protein, according to claim 1, which consists of SEQ. ID NO:12.

5. The isolated soluble fusion protein, according to claim 2, which consists of SEQ. ID NO:13.

6. The isolated soluble fusion protein, according to claim 3, which consists of SEQ. ID NO:14.
- * * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 9,273,137 B2
APPLICATION NO. : 13/842345
DATED : March 1, 2016
INVENTOR(S) : Fang et al.

Page 1 of 2

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

Column 1,

Line 45, "(HGF) etc." should read --(HGF) etc.--.

Column 2,

Line 40, "Prof Hanahan's" should read --Prof. Hanahan's--.

Column 3,

Line 51, "a third ig-like" should read --a third Ig-like--.

Column 5,

Line 30, "Fe region" should read --Fc region--.

Column 12,

Line 63, ", edition," should read --, 2nd edition,--.

Column 14,

Line 33, "2" edition" should read --2nd edition--.

Column 20,

Line 24, "B 16 melanoma" should read --B16 melanoma--.

Column 26,

Line 3, "300 PBST" should read --300 μ L PBST--.

Column 26,

Line 31, "deter mined" should read --determined--.

Signed and Sealed this
Twenty-first Day of June, 2016



Michelle K. Lee
Director of the United States Patent and Trademark Office

CERTIFICATE OF CORRECTION (continued)

Page 2 of 2

U.S. Pat. No. 9,273,137 B2

Column 29,

Line 11, “[12] Donerrell MI” should read --[12] Dorrell MI--.